World Protein Resources

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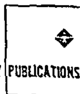
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FOREWORD

ADVANCES IN CHEMISTRY SERIES was founded in 1949 by the American Chemical Society as an outlet for symposia and collections of data in special areas of topical interest that could not be accommodated in the Society's journals It provides a medium for symposia that would otherwise be fragmented, their papers distributed among several journals or not published at all Papers are refereed critically according to ACS editorial standards and receive the careful attention and processing characteristic of ACS publications Papers published in ADVANCES IN CHEMISTRY SERIES are original contributions not published elsewhere in whole or major part and include reports of research as well as reviews since symposia may embrace both types of presentation

CONTEN

PREFACE

In the totality of the problem of overcoming the world food shortage, providing sufficient protein occupies a special place, first because it affects primarily children—not only as children in terms of their survival, but also in terms of their growth and development Evidence is accumulating that protein deficiency during the preschool years could result in retarded mental and physical development This problem also occupies a special place because it is becoming clear that to produce the amounts of protein concentrate needed, it will not be enough to extend the present "classical" methods of producing animal protein, there will be the need to develop and exploit other sources of protein concentrate ranging from fish and oilseeds to petroleum

It is not so easy to estimate accurately the extent of the problem Abbot concludes (p 13)

> The longer term view, taking account of expected population increases to 1975 and beyond, opens up a truly immense demand for protein To eliminate malnutrition would mean a 45% increase in total protein supplies for the period 1960–1975 Allowing also for a hoped for annual income growth of 5% in the developing countries, the demand in the year 2000 will be 2 1/2 times that of 1960 More than twice as much protein will still be needed even if there is no improvement in diets

Some have other ways of presenting the extent o the problem A considerable literature is building up on this subject (*1, 2, 4, 5*) All agree that this is a most serious problem

It is not uniformly recognized that we do not now have all the means of solving this problem It has been said often that if all the accumulated knowledge of food and agriculture were applied to the present problems of food deficiency these could be solved (*3*) All that is needed is to raise the economic level to modernize farming to educate the people, and to industrialize No one can quarrel with this point of view If all this could be done the problems would be solved and it is happening in some selected countries But it is not happening rapidly enough in the places where the need is the greatest And there is the question of whether it can be done at all in that way in many places

There is a certain soporific quality about this idea the contention that there s no need to think of new approaches to food—particularly protein food It is suggested that one merely transfers what is already known, the problems of the protein poor countries are considered to be no different qualitatively from the problems facing the industrialized countries

However, the question of competency to solve the protein problem is an open one, we can examine with profit our current methodology and thinking and judge whether our attacks on this problem are consistent with the state of our accumulated knowledge and technology It is appropriate that this be done at a meeting of the American Chemical Society After all, this is basically a problem in chemistry, can we, through the various aspects of chemical manipulation, provide the world with sufficient protein? Therefore, in the background is the question, "What can chemical science contribute to the enhancement of the present protein forms and to the development of new protein concentrates?"

It is also appropriate that we dedicate this meeting to the memory of Maurice Pate of UNICEF He and his agency distributed milk protein to women and children all over the world They established the value of protein concentrates and the validity of the special character of the protein deficiency problem, they pioneered in recognizing the need for protein concentrates from new sources and stimulated research and development on new protein food forms

If our general analysis is correct, we can expect that the solutions will be manifold, dictated by national and regional capabilities, and local conditions and cultures There probably will be areas of the world where mere application of the present knowledge will be sufficient, but in many other places radical new approaches will be necessary We have brought together at this symposium a sampling of the wide range of possibilities We are evaluating the possibilities of the classical sources of protein concentrates—meat and milk We are looking at the status and possibilities of seed proteins, fish, and some of the newer ideas

I detect a sense of urgency about the world protein food problem It is not improving with time and the chances of its worsening are good It is the hope of those who organized this symposium that this exchange of information and ideas will improve chances of specific solutions and accelerate development of a general solution to this problem

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New Orleans, La

AARON M ALTSCHUL

October 1965



Maurice Pate

Maurice Pate: 1894-1965

Maurice Pate, Executive Director of the United Nations Children's Emergency Fund (UNICEF) since its founding in 1946 until his death on January 19, 1965, was a practical humanitarian and, in his characteristically unassuming way, a stubborn idealist.

Mr Pate has been called the foster father to tens of millions of children of every race and nationality. A Phi Beta Kappa graduate of Princeton University (Class of 1915), Mr Pate was UNICEF's first Executive Director. Under his direction UNICEF became a world wide operation assisting 118 nations in more than 510 programs for children. A tireless traveler, he visited almost all of the 118 countries in which UNICEF aids projects affecting the health, nutrition, and education of children. "It's inspiring," he once said, "to see children more alert, with healthier bodies, in part because of our aid."

Born in Pender, Neb., on October 14, 1894, Mr Pate began his career in a small country bank in Iowa, but World War I awakened his zeal for public service. He soon volunteered for work with the Commission for Relief in Belgium under its chief, Herbert Hoover, who remained a long time colleague and close friend. Following a year's service in the U.S. Army in France, Mr Pate directed the Child Feeding Program of the American Relief Administration, which provided a daily meal to 1,300,000 Polish children. His administrative ability led him to work in Poland for the Standard Oil Co. of New Jersey, and to start his own private import and banking business.

Once again world crisis summoned him to public service, and he served as president of the Commission for Polish Relief from 1939 to 1941. A year later he became director of the American Red Cross Relief to Prisoners of War. In his five years in office he succeeded in moving $170,000,000 in relief supplies to aid American and Allied prisoners of war.

While making a world food survey with Herbert Hoover following World War II, Pate was struck by the terrible conditions affecting children. The report of this survey was a moving factor in the creation of UNICEF by the United Nations General Assembly in 1946. The first private contribution to UNICEF—a check for $2000—was made by Pate himself.

Mr Pate carried in his wallet a slip of paper with a quotation that summarized his philosophy of life: "A true reformer," it read, "is one who recognizes in human nature a higher quality than its average performance." His extraordinary ability to find a "humanitarian consensus" that cut across

national and political lines reflected his faith that people all over the world could best be reached by appealing to the nobler impulses of their common humanity He would have desired only one tribute to his memory the rededication of his friends and colleagues to the cause of children and to the cause of international cooperation

Modesty, dignity, and quiet determination characterized everything Mr Pate did He learned the art of being a practical humanitarian under the most difficult conditions In occupied Belgium, during the First World War, and in Eastern Europe during the chaotic years that immediately followed, profiting by the inspiration and tutelage of Herbert Hoover, he discovered the power of disinterested goodwill, sometimes managing to obtain the cooperation of governments that were actually fighting each other to get shipments of food and medicine through to devastated areas

Mr Pate's 18 years with UNICEF were also years of considerable international tension, but he succeeded in gaining the cooperation of governments all over the world by his firm determination that UNICEF should be absolutely above international politics "My personal position is simple," he stated "I believe in universality based on the factor of needs I believe in extending relief to children without distinction, either within countries or between countries" A true international civil servant, he insisted that UNICEF's mandate to deal with the outstanding needs of children, regardless of their own or their parents' race, creed, nationality status, or political belief, be respected to the letter

Under Mr Pate's leadership, UNICEF evolved from a relief organization into one of the most effective instruments yet devised for long term aid through international cooperation UNICEF supplies and equipment, the training programs it aids, and the over-all advice and stimulus it has given have enabled 133 countries or territories to launch and strengthen vital programs to improve the health, nutrition, and education of the rising generation Always foremost in Mr Pate's mind was the question, "What can we do? What action can we take now?" In accordance with his pragmatic approach, UNICEF aid took whatever form was needed to unlock local resources It might be bicycles to enable midwives to make their rounds more quickly, educational toys for a model kindergarten, a printing press to produce primary textbooks, or the equipment for an entire milk plant

As a result of his persistent efforts, the number of governments pledging voluntary contributions to UNICEF increased from 35 in 1951 to 121 (including the U S A) in 1964 He spoke extremely slowly and in a quiet voice Though appealing to the conscience of humanity, he was not a sentimentalist He would merely ask, "How can we build a peaceful and prosperous world without healthy, well nourished youngsters who have been trained to contribute to their countries' development?" His argument was that sick children, hungry children, children deprived of schooling were

not only a burden on humanity's conscience but a criminal waste of essential human resources

Although he had no children of his own, Mr Pate could appreciate the tragedy of families where a bare majority of children survive to reach school age The eldest of seven children, he saw three of the others die in infancy—one of polio, one of diphtheria, and one of an intestinal infection brought on by drinking unpasteurized milk He once remarked to a friend, 'They all could have been saved if we'd known then what we know today " With what we know today, he always pointed out, countless children in the developing countries, now doomed to hunger, sickness, and ignorance, could be saved to build a better world Maurice Pate dedicated his life to this task, and the millions of children who have been protected from crippling disease and given a better chance in life through the intervention of UNICEF are the most fitting memorial he could have desired

His work for UNICEF and the world's children took Pate to the offices of presidents and prime ministers, leaders and kings, as well as to the papal apartments of Popes Pius XII John XXIII, and Paul VI

Because of his reputation as an expert on food distribution, the late United Nations Secretary General Dag Hammarskjold appointed Maurice Pate as his special representative during the Congo crisis in 1960 to open up emergency food distribution

His work for children was the focus of Maurice Pate's life For these efforts he earned a host of decorations, including an honorary doctorate of philanthropy from Princeton University in 1958 the Albert and Mary Lasker Award in 1959 for notable service in improving child and mother welfare as well as other awards The governments of Belgium, France, the Netherlands, Poland and other countries decorated him In 1960, the Norwegian National Committee for UNICEF suggested Pate be nominated for the Nobel Peace Prize He said he would not accept such an honor for himself suggesting that UNICEF itself be nominated

Five U S Presidents—Johnson Kennedy Eisenhower Truman, and Hoover—have hailed UNICEF s work under Maurice Pate s leadership Lyndon B Johnson said UNICEF's helping hand has been extended to children in the underdeveloped countries of the world Even in this era of highly advanced science and technology three out of four of all the world's children today still live in the shadow of poverty, hunger, and disease ' John F Kennedy said, 'Through the years the efforts of UNICEF have met with a remarkable measure of success ' Said Harry S Truman I have followed with satisfaction the humanitarian services performed by UNICEF in many countries the world over while Dwight D Eisenhower commented 'It is heartwarming to follow the work of UNICEF which contributes much to the lives of millions in need '

The late Herbert Hoover made this assessment of the unassuming tireless persistent man who led UNICEF for so long ' He has given his

life to restoring life and chatter among the world's children the most efficient and dedicated human angel that I have ever known "

United Nations Secretary-General U Thant said, "The credit for UNICEF's unique achievement belongs to Maurice Pate He was truly a great humanitarian "

Donald R Sabin, *Director*
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United Nations New York

August 6, 1965

EDITOR'S NOTE In October following the symposium it was announced that the Nobel Peace Prize for 1965 would be awarded to UNICEF I, speaking about Mr Pate in connection with his announcenment, U Thant Secretary-General of the United Nations, said that "in a sense, this is a posthumous tribute to his selfless work as Executive Director of UNICEF for so many years "

INTRODUCTIONS

This Symposium on Evaluation of World Protein Resources deals with proteins from plants, both the higher green plants and the lower plant forms, bacteria and fungi. Fifteen of the 22 papers are devoted to plant proteins. This emphasis on proteins from plants is proper for plants are the primary and greatest source of amino acids and proteins. Almost all of the essential amino acids which animals, including man, consume are initially produced in plant cells, most frequently in the cells of green leaves and must generally be concentrated into proteins of seed or tubers or converted into animal protein before they can be used by man.

In my estimation we should greatly increase our research on all forms of plant proteins and ways of concentrating and converting them into edible and acceptable forms. In "the horse and buggy days" we used to feed grain and forage to animals to convert the stored energy into animal power useful for work or transportation. However, scientists found other ways to convert and utilize different forms of energy, including that in fossil fuels and the primary ones of fission and fusion.

I wonder if the conversion of plant proteins into animal proteins may not some day be considered a remnant of the horse and buggy days in which animals were used to concentrate the protein from plants. The challenge that faces the protein chemist today is to find ways to use new, untapped sources of protein which may in the end be as important to a protein hungry humanity as was developing and utilizing new forms of energy.

Plant biochemists and geneticists have cooperated to search for and find mutants in maize that produce protein of a much better quality. This growing corn with a higher lysine content may have an impact upon agriculture and human nutrition in some countries which will be as important as the introduction of hybrid corn.

Methods have been developed for processing lower plant forms such as bacteria to produce protein concentrates. Microorganisms including bacteria have been proposed as a potential source of protein, but much remains to be done to develop suitable processing techniques for producing a high quality protein from this little explored source.

Fungi represent another lower plant form which might contribute to the world protein supply Protein production from mold has potential for a fungal protein process

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September 1965

The poor peoples of the world do not get enough good protein, and the deficit is especially serious in young children Rapidly growing children have greater protein requirements than adults and yet are fed even more exclusively than adults on the local starchy food, usually a grain The grains have enough, or almost enough protein but are deficient in certain amino acids The main problem, then, is how to add the missing amino acids

This is precisely the problem faced in formulating feeds for non-ruminant animals Animal feeds must be based on locally available materials, be nutritionally complete, and be as cheap as possible The same requirements must be met in foods for poor, underfed children, and in addition these foods must be made safe and acceptable at as little extra cost as possible Similar requirements lead to similar solutions Therefore, cheap adequate foods for underfed children are of the same materials and the same general technological character as the proved animal feeds In all feed and foods, adjustments in composition must be made to meet specific nutritional needs

In animal feeds, and in the related foods for children, the protein of grain is upgraded by adding fish or oilseed materials which are concentrated in protein and provide the amino acids lacking in the grain

An alternative approach to fish and oilseed protein concentrates, which will become more and more important in time, is to upgrade the proteins of grains, not with protein concentrates, but, either completely or partially, with industrially produced amino acids

Present knowledge of protein nutrition, although less complete so far as children are concerned than nutritionists often pretend, is adequate for going ahead in a big way Technological knowledge about producing suitable protein-adequate foods, although even less complete than nu-

tritional knowledge, is not the main bottleneck The main bottleneck is in marketing in solving the social and economic problems involved in getting protein adequate foods produced, sold, and used, and in getting the foods into the normal internal economy through normal commercial channels

In the richer countries of the world, more expensive processing of cheap protein materials can be considered, with the object of obtaining more attractive foods similar in general character to the much appreciated animal protein foods And it is now basically understood how to achieve in a factory the sort of transformation of crude plant materials into attractive protein foods which is achieved by processing in the animal A technical revolution is one of man's major ancient activities The main technical problem is how to obtain desirable structure from a mushy precipitate of isolated protein

Finally, the protein upgrading of foods for underfed children has so far made only a trivial quantitative impression on the satisfaction of the global needs Structured products are as yet of trivial commercial importance in the markets of the richer countries We are just at the starting point of great developments

For a dozen years or so a relatively small group of interested men has understood the protein needs of underfed children and the general technological approach to satisfying these needs More than a dozen years ago some basic ways of producing structured foods from plant protein materials were worked out, but interest in the field was limited to laboratories, and to very few of these Recently and rather suddenly, the picture has changed A symposium such as this one, which reflects very broad interest in its subject matter, would have been unthinkable not long ago And who would have predicted not long ago that our foreign food policy would veer from primary concern with the mere disposal of surplus crops to an emphasis on satisfying human nutritional needs, that it would be declared government policy to help with the protein upgrading of grains especially for foods for children in the undeveloped countries? So far as structured plant protein foods are concerned work is going on in various large industrial research laboratories and products are at last beginning to appear on the market Further rapid progress is now assured

Thus for the problems and products discussed we are proceeding from understanding and preliminary work of all kinds to interest and development and action on a large scale We are at the exciting take-off point The timing of our discussion, therefore is peculiarly well chosen

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September 1965

Protein Supplies and Prospects: The Problem

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While animal protein resources are rising in the developed countries, in the developing countries the total per capita protein supply has declined by about 6% since World War II, with increased dependence on protein from grain. On an average, present supplies are generally adequate for nutrition in practice, variations in individual requirements and economic and social impediments to distribution mean that substantial segments of the population do not receive enough The prospects of meeting these needs through protein from cereals, oilseeds, pulses and nuts, fruits and vegetables, livestock products, fish, and less conventional sources of protein are appraised, with indications of relative cost Immediate deficiencies could be met by specific programs to correct distributional discrepancies To cover future requirements massive increases in protein food production must be envisaged

This paper introduces an evaluation of protein resources and requirements on a world basis It attempts first to summarize available data on the supply of food protein then appraise these supplies in terms of the quantities and per capita distribution necessary for adequate nutrition Then the prospects of expanding the supply of protein from different sources are reviewed and some of the economic issues identified

Supplies of Food Protein

Table I sets out the quantities of protein obtained from various groups of foods in the major regions of the world These figures are recent estimates of production adjusted by net trade and allowances for seed livestock use waste etc divided by the estimated total population Some 70% of the world's protein supply comes from vegetable sources and 30%

from animal sources. The penultimate two lines of Table I are a measure of the contrast between the situation in the well-fed parts of the world and the others. Protein supplies through grains are strikingly similar on a per capita basis. In contrast, availabilities of protein from meat and dairy products are of the order of 20 grams daily per capita, respectively, as against 3 to 4 grams in the developing countries, with only a somewhat higher supply from pulses and oilseeds as a partial compensation.

Grains, the staple food in most countries, are also the main source of protein, furnishing almost half the total supply. This proportion ranges from 72% in Pakistan to 16% in the United States (Table II). Grains are the main source of protein in all countries except Argentina, Australia, Canada, New Zealand, and the United States (where it is meat) and Ireland and Sweden (where dairy products are more important).

Pulses, oilseeds, and nuts provide almost 13% of the world's protein supplies. Their contribution is as high as 27% in India and probably of the same order in mainland China. They provide 23% of the protein in Brazil and 19% in Japan and Mexico, but in most countries much less.

Starchy roots account for about 5% of protein supplies, rising to 12% in Peru, and perhaps even more in those African countries where cassava or yams are the staple food.

Vegetables and fruits provide only about 3% of the world's protein, their contribution rises to 9% in Spain and 8% in Libya, Peru, the Philippines, and Portugal.

Among animal products, meat and dairy products are of almost equal importance in the world's protein supply, the former contributing about 13% and the latter about 11%. Meat ranges from 44% in Argentina to 2% in Ceylon, India, and Pakistan, and dairy products from 35% in Finland to 1% in Japan.

Although eggs provide only about 2% of the world's protein, they amount to as much as 7% in Israel and the United States.

Fish too accounts for only about 3% on a world basis but is the main source of animal protein in countries such as Ceylon, Japan, the Philippines, Portugal, and China (Taiwan). In Portugal fish supplies 23% of the total protein and in Japan, 18%.

Supply trends on a world basis become evident only slowly. In the economically more developed countries protein intake has increased by some 6% since before World War II, in the developing countries where increases are most needed, there has been a decline of about 6%. The main change in the composition of protein supplies since before the war has been a reduction in the protein derived from grains in the better-off countries and a large increase in that obtained from animal sources.

Table I Protein Supplies Per Capita by Major Food Groups and Regions (3)

	Vegetable Protein				*Animal Protein*				
	Grains	*Starchy Roots*	*Pulses Oil seeds and Nuts*	*Vegetables and Fruit*	*Meat and Poultry*	*Eggs*	*Fish*	*Milk and Milk Products*	
	Grams per Day								*Total*
North America	15.7	2.3	4.7	4.6	31.9	6.0	2.5	25.3	93
Australia and New Zealand	24.3	2.4	2.1	3.1	36.8	3.5	2.2	19.5	94
Western Europe	30.5	4.4	5.0	4.1	16.2	3.1	2.4	17.3	83
Eastern Europe and USSR	48.3	8.2	2.0	2.5	12.9	2.2	1.9	16.1	94
Latin America	26.5	2.7	10.7	2.8	13.8	1.2	1.5	7.4	67
Far East	32.2	1.8	12.0	1.7	3.0	0.4	2.2	2.2	56
Near East	48.5	0.7	9.5	3.6	4.6	0.5	1.1	7.4	76
Africa	32.2	7.1	9.0	1.7	5.8	0.4	1.3	3.5	61
Europe North America Australia New Zealand Argentina Paraguay Uruguay	33.4	5.2	3.8	3.6	19.8	3.3	2.4	18.5	90
Far East Near East Africa Latin America (except Argentina Paraguay and Uruguay)	33.2	2.3	11.6	1.8	3.8	0.4	1.9	2.9	58
World	33.4	3.2	9.0	2.4	8.8	1.2	2.3	7.7	68

Adequacy of Present Level of Supplies

The Joint FAO/WHO Expert Group on Protein Requirements, in October 1963 recommended the following broad basis for assessing protein requirements (grams per kilogram of body weight per day according to age group) years 1 to 3 0.9 gram 4 to 9 0.8 gram, 10 to 15 0.7 gram, 16 upward 0.6 gram with due allowance for the value of the type of food protein

Average protein requirements estimated accordingly vary from 40 grams per capita per day in the United States to 52 grams in Yugoslavia for example This reflects in part the age sex and body weight of the population but principally the biological quality of the protein as measured in terms of net protein utilization (NPU) or protein efficiency ratio (PER) Good quality proteins are widely present in animal products such as milk, meat fish, cheese, and eggs and in the seeds of many legumes and pulses Starchy foods such as cassava and plantains and to a large extent maize

and wheat, contain protein of relatively poor quality Estimates of requirements in a number of countries are contrasted with available supplies in Table III The low requirement in the United States results mainly from the high quality of the dietary protein, while in the developing countries the requirements are higher because protein quality is lower

On an average basis available supplies exceed requirements in most of the countries for which there are data However, national average figures do not reveal the real situation so far as individuals are concerned For two sets of reasons these averages cannot be accepted as satisfactory Because of individual variations in requirements 20% more than the average is recommended to cover the needs of most of the population (*5*) Economic and social factors further impede the allocation of supplies according to physiological needs Thus, in India lower income groups in the urban areas of Maharashtra, for example, amounting to between a quarter and a third of the households do not receive enough protein (*13*) The proportion of persons with inadequate protein supplies would be still higher since in households where food supplies are limited the productive members or wage earners may take all the protein they need and more, at the expense of the nutritionally vulnerable groups—children, and expectant and nursing mothers Surveys of rural households in Uttar Pradesh indicate similar deficiencies (11) Consequently, considerable proportions of the population probably receive inadequate supplies of protein in countries such as Colombia, Peru, Ceylon, India, Pakistan, and the Philippines, and in various parts of Africa, where total average requirements seem to be just covered Similarly, while it is probable that even on a world basis the total available supplies of protein are enough to satisfy the calculated requirements of the whole population, in actual fact many people consume much more protein than they need, supplies must be sufficient to take account of this as well as to satisfy minimum requirements Thus, with distribution according to effective demand, supplies considerably in excess of the calculated average requirement will be needed The amount of the excess needed will differ from country to country, according to the degree of income disparity family attitudes as to food appropriate for women and children, spoilage during preparation, availability at the proper time and place, etc

Prospects for Protein Supplies

Grains Grains provide nearly half of man's total supply of protein directly, plus much of that obtained via livestock products As staple foods providing the bulk of the calorie supply, grains will continue to be the main sources of protein in the foreseeable future Since almost half the present supply, including by-products of processing for human consumption, is fed to livestock, and output in some of the most productive

Table II Per Capita Protein (Averages

	Total Protein	Animal Protein	Grain	Starchy Roots
U S A	92			
Grams per day		65	15	2
% of total protein		70	16	2
Canada	95			
Grams per day		63	23	3
% of total protein		66	24	3
Argentina	98			
Grams per day		57	34	3
% of total protein		58	35	3
Brazil	61			
Grams per day		19	24	2
% of total protein		31	39	3
Colombia	48			
Grams per day		23	15	2
% of total protein		48	31	4
Mexico	68			
Grams per day		20	33	—
% of total protein		30	49	—
Peru	49			
Grams per day		12	22	6
% of total protein		24	45	12
Ireland	96			
Grams per day		57	29	7
% of total protein		59	30	7
Finland	94			
Grams per day		53	34	5
% of total protein		56	36	5
Portugal	72			
Grams per day		26	29	6
% of total protein		37	41	8
Sweden	61			
Grams per day		52	22	4
% of total protein		64	27	5
Israel	84			
Grams per day		33	39	2
% of total protein		40	47	2
Libya	53			
Grams per day		10	34	1
% of total protein		20	6	2
Turkey				
Grams per day			6	2
% of total protein				
United Arab Republic				
Grams per day			5	
% of total protein			6	

meat imports from her northern neighbors and from overseas to reach a consumption of 20 pounds per person per annum, as against only 2.2 to 4.4 pounds in nearby Liberia and Sierra Leone. With cocoa prices declining and public nonfood expenditure augmented, these imports have been cut back.

While there are parts of Africa, for example, with good potential for increased livestock production, the pest and disease control measures and changes in management practice required are formidable. It is unlikely that meat production in the developing countries can be increased very rapidly in the immediate future. Prospects of expanding the supply and consumption of poultry meat are perhaps rather brighter than for other meats, given the high feed conversion productivity of modern broiler strains and the general acceptability of chicken meat. However, at present there are no signs of production costs falling below the U.S. level, which means that poultry meat will still be an expensive protein to low income populations. This also applies to eggs, though improvements in the type and management of birds kept on a scavenging basis could help at the local level.

Fish. Marketing problems limit the consumption of fish in many parts of the developing world. However, dried fish is a major source of protein in tropical Africa, as are fermented and dried fish in parts of Asia. Fish and new fish protein concentrates can be expected to compete advantageously with other protein foods up to the limit of the supply. Since some 10 of the estimated 45 million tons of fish caught at present are reduced to meal for animal feeding, there seems no immediate likelihood of a shortage. However, to get these fish to potential consumers would involve major investments in marketing equipment, some extra costs, and the important question of foreign exchange availability.

The long-run prospects for fish as a protein source are of particular interest, both because it competes less directly with other food for productive resources, and because the political and social issues involved in expanding supplies may be much less complicated than for livestock, for example. The potential world harvest of fish, using the traditional hunting-type methods of exploitation, and without endangering known stocks, has been estimated conservatively as approximately 115 million tons, or about 2.5 times the present level *(9)*.

Processed Plant Protein Concentrates. While protein from soybeans prepared according to traditional methods has maintained dietary standards in the Far East for centuries, the use of new protein-rich foods based on these sources elsewhere is still very limited (Table V).

It is essential that such products appeal to the consumer and be capable of introduction into existing marketing channels in such a way

meat imports from her northern neighbors and from overseas to reach a consumption of 20 pounds per person per annum, as against only 2 2 to 4 4 pounds in nearby Liberia and Sierra Leone With cocoa prices declining and public nonfood expenditure augmented, these imports have been cut back

While there are parts of Africa, for example, with good potential for increased livestock production, the pest and disease control measures and changes in management practice required are formidable It is unlikely that meat production in the developing countries can be increased very rapidly in the immediate future Prospects of expanding the supply and consumption of poultry meat are perhaps rather brighter than for other meats, given the high feed conversion productivity of modern broiler strains and the general acceptability of chicken meat However, at present there are no signs of production costs falling below the U S level, which means that poultry meat will still be an expensive protein to low income populations This also applies to eggs, though improvements in the type and management of birds kept on a scavenging basis could help at the local level

Fish Marketing problems limit the consumption of fish in many parts of the developing world However, dried fish is a major source of protein in tropical Africa, as are fermented and dried fish in parts of Asia Fish and new fish protein concentrates can be expected to compete advantageously with other protein foods up to the limit of the supply Since some 10 of the estimated 45 million tons of fish caught at present are reduced to meal for animal feeding, there seems no immediate likelihood of a shortage However, to get these fish to potential consumers would involve major investments in marketing equipment, some extra costs, and the important question of foreign exchange availability

The long run prospects for fish as a protein source are of particular interest, both because it competes less directly with other food for productive resources, and because the political and social issues involved in expanding supplies may be much less complicated than for livestock, for example The potential world harvest of fish, using the traditional hunting-type methods of exploitation, and without endangering known stocks, has been estimated conservatively as approximately 115 million tons, or about 2 5 times the present level (*9*)

Processed Plant Protein Concentrates While protein from soybeans prepared according to traditional methods has maintained dietary standards in the Far East for centuries, the use of new protein rich foods based on these sources elsewhere is still very limited (Table V)

It is essential that such products appeal to the consumer and be capable of introduction into existing marketing channels in such a way

as to reach consumers who are not otherwise getting enough protein A difficulty is that commercial feed grade oilcake is not usually a suitable raw material for human food Special processing is often necessary and also careful selection and handling of raw material to avoid developing toxic substances and to remove or minimize indigenous biologically deleterious compounds Consequently edible protein concentrates from oilseeds packaged for commercial distribution tend to cost several times as much as commercial feed grade oil cake though they remain a relatively cheap source of protein (*1*)

Table III Protein Requirements and Supplies Available
(Grams per capita daily) (*3 6*)

	Requirements	*Supplies*
U S A	40	92
Canada	42	95
Australia	45	92
New Zealand	44	105
Britain	44	86
France	47	96
Germany Federal Republic	44	79
Greece	49	95
Ireland	45	96
Italy	46	77
Sweden	48	81
Switzerland	44	90
Yugoslavia	52	96
Argentina	42	98
Brazil	45	61
Chile	46	77
Colombia	48	48
Mexico	44	68
Peru	48	58
Ceylon	47	45
China (Taiwan)	42	57
India	48	51
Japan	43	67
Pakistan	46	46
Philippines	46	47
Israel	44	83
Libya	47	53
Turkey	45	90
U A R	45	76
Mauritius	42	46
South Africa	41	73

Table IV Protein Intake Daily Per Capita and Potential Oilseed Availability Illustrative Countries (1960 62) (3, 4)

Countries	Population (Millions Average) (1960–62)	Animal Proteins, G	Oilseed Proteins, G	
			Actual	Potential on Basis of Production
Mexico	36 1	23	1 4	15 8
Brazil	71 8	18	3 3	15 7
Colombia[a]	14 3	20	0 4	7 3
Peru	9 9	14	0 1	11 6
U A R	26 9	12	0 6	15 8
Nigeria	50 0	5	1 8	14 6
Pakistan	95 8	8	—	5 3
India	450 5	6	0 6	8 8
Ceylon	10 2	8	2 5	15 3
Philippines	28 3	14	1 1	8 8
China (Taiwan)	11 7	15	6 2	8 5

[a] 1960–61

Protein Isolates These products tend to be expensive, and there are still insufficient data for predicting large-scale use in the future However, their incorporation in ice cream, as in Japan, is seen as providing an initial basis for commercial processing and marketing operations in a country like India It might then be easier to develop less remunerative but more nutritionally desirable lines, possibly with government assistance

Unconventional Sources of Food Protein Food yeast can be produced in large quantities at comparatively low cost There are large-scale facilities in several countries, but because of difficulties in making it attractive, its use in human nutrition has met little success

Problems in using green leaves as a protein food include both color and taste as regards consumer acceptability and also variability in nutritive value So far no satisfactory and economic source of suitable raw material has been found

Algae such as *Chlorella*, and proteins synthesized by solar energy or produced from yeast grown on paraffinic hydrocarbon fractions obtained from petroleum appear to be promising sources Here again the problem is one of acceptability It may well be that such sources of protein will provide large quantities of animal feed in the future This could both reduce the cost of livestock products from human consumption and release for human use protein feed that would otherwise have been consumed by livestock

Since cost is the main limiting factor for the population groups with inadequate diets, it must be kept to the forefront of any appraisal of alternative protein sources A cost comparison of the protein in various foods is attempted in Table VI It is understood, of course, that the prices used reflect other attributes of these foods as much as protein content Furthermore, important duties and restrictions, transport costs, distribution margins, etc , can change radically the cost interrelationship in a particular country

Table V Consumption of Oilseed Protein Concentrates in Some Main Consuming Areas

(Thousand Tons)

Miso Tofu Tempeh and Similar Fermented or Cooked Soybean Products		*Soybean, Peanut Cotton seed Flour etc for Mixing with Other Foods*		*Soybean Milks*	
China (Mainland)	5 000	U S A	245 0	Singapore and Malaya	1 3
Japan	2 447	Japan	60 0	Hongkong	1 0
Indonesia	385	Britain	40 0		
China (Taiwan)	209	South Africa	1 8		
Korea (South)	158	Indonesia	0 4		
Hongkong	15				
Malaya	15				
Singapore	15				

Scope of Problem

✓ The dimensions of the immediate protein malnutrition problem are set by the size of the vulnerable groups especially children, in low income populations Several approaches to a solution can be envisaged devising and teaching ways by which more protein is obtained from food already eaten as, for example, reducing processing and cooking losses in grains, pulses and vegetables, marketing new protein foods or traditional foods incorporating protein supplements that are attractive, convenient, and inexpensive distribution on a subsidized basis to selected groups through schools, etc Increasing awareness on the part of governments of what can be done by specific well directed programs, better knowledge of the techniques and resources needed, and willingness of other governments and international agencies and enterprises to help could solve this problem

The longer term view taking account of expected population increases to 1975 and beyond (United Nations ' medium assumption" population projections) opens up a truly immense demand for protein To eliminate malnutrition would mean a 45% increase in total protein supplies between 1960 and 1975 Allowing also for a hoped for annual income growth of 5%

Table VI Cost of Protein Content of Various Foods

(Wholesale on major world markets)

Product	*Protein, %*	*Price of Product, Cents/Pound*	*Price of Protein, Cents/Pound*
Skim milk powder	36	13 5	37
Fish			
Dried[b]	37	14	39
Flour[c]	80	18	23
Beef[d]	12	18	155
Chicken[e]	15	25	165
Egg[f]	11	24	211
Chick peas (gram)[g]	20	6	28
Beans[h]	22	8	35
Wheat flour	12	4	31
Defatted soybean flour[i]	*51*	*9*	*18*
Protein isolate, soybean[j]	97	43	44

[a] Commercial exports average price, f o b 1963–64
[b] Angola 1960
[c] Deodorized, defatted Morocco
[d] Australia export sides frozen Average 1961–63 protein percentage calculated as for Australian medium fat dressed carcass
U S ready-to-cook broilers, price 25 cents per pound Protein ratio from (*14*)
[f] Average monthly price, Danish Export Co-operative 1963
[g] Banda, Uttar Pradesh, India, February 1964
[h] Dry, Mexico City, December 1963
For bakery and other processing uses in Japan and U S A
[j] Average of U S range (*2*)

in the developing countries, the demand in the year 2000 will be two and a half times that of 1960 More than twice as much protein will still be needed, even if there is no improvement in diets (7) With such a prospect the scope for reorganizing plant and animal production, processing, and marketing, for applying technology and capital, for watering dry areas, and for cultivating the sea, is great indeed

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2

General Outlook for Animal Protein in Food Supplies of Developing Areas

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According to history, as the economic level of a given population improves, the consumption of foods from animal sources increases. The animal is not an efficient converter of feed to food and adequate diets may be supplied at lower cost from plant sources. However, this requires a high degree of sophistication in both knowledge and food supplies, neither of which is evident in areas of serious protein deficits. There is a real opportunity to develop animal culture with increased efficiencies, and to utilize feed materials now considered inedible although there will be competition with humans for some portions of the food supply. Therefore, we may expect animal protein supplies to be increased along with protein from plant sources.

THE inadequacy of our total food supply, world-wide, has been well documented. Likewise, available data indicate that the supply of edible protein, world-wide, is entirely inadequate to meet the needs of the present population and must be increased very considerably to fill the needs of the increasing population that is inevitably before us.

We are discussing here the protein supplies of the world, but realistically we cannot separate any one food constituent from all others when we are considering world food supplies. We must not lose sight of the fact that when calories are inadequate, some of the protein is going to be used to supply energy. We must also take into consideration the fact that all proteins are not equally adequate to meet the nutritional requirements and that there is a difference in the availability of amino acids from protein to protein.

It is with these concepts in mind that we approach the question of the role of animal proteins in helping to meet the food needs of the future. It is recognized at the outset that from the stand point of pure economics we can supply diets that will sustain life more economically by direct use

of protein material than by converting it to animal protein However, as Wright (*14*) has pointed out, many animals do not compete with the human for the type of food they eat The ruminants, particularly, convert grass and grass products and other forages into more readily available and certainly more desirable forms of protein

The protein from these sources is not only concentrated to provide a higher protein content of animal protein in the carcass of the animal, but much protein is added through the conversion in the rumen Even where the animal may be competing with the human for certain types of food, as may be the case with the pig and the hen, these animals are producing a product desired by man The 1965 estimated total production of meats for the 44 major producing countries is estimated to be 50 3 million tons (*12*)

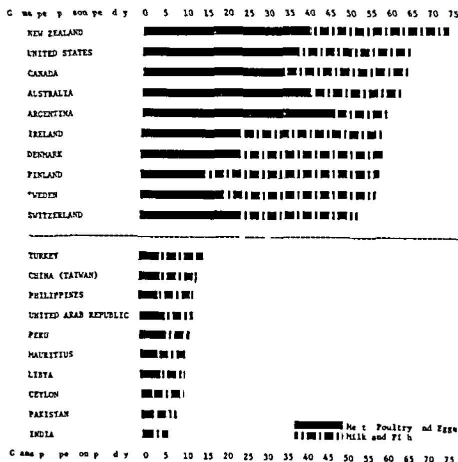


Figure 1 Available total edible protein supplies

Phillips (*9*) has presented data which show the total protein supplies in grams per person per day for 43 countries and also the total animal protein by source available for those same countries We have rearranged these data for the 10 high and 10 low countries in Figure 1 to show the available

protein in grams per person per day supplied by meat, poultry, and eggs, which are the subject of this particular discussion

The ten countries low in supplies of animal protein available for human consumption provide from 5 to 14 grams per person per day, in contrast to 51 to 74 grams in countries where edible animal protein is more plentiful The difference between the part of these totals produced from meat, poultry, and eggs is also striking, ranging from 2 to 6 grams in the lower group to 14 to 44 grams in the higher group The balance of these animal protein supplies, of course, is provided by fish and by milk and milk products

The total edible protein in grams per person per day for these same selected countries is presented in Figure 2 The ranges in total protein supply for the ten countries low in dietary animal protein are from 45 to 89 5 grams per person per day, whereas in the ten countries high in animal protein, it is from 60 to 105 grams All of the high ten, except Sweden, have more than sufficient total supplies to meet the amounts recommended

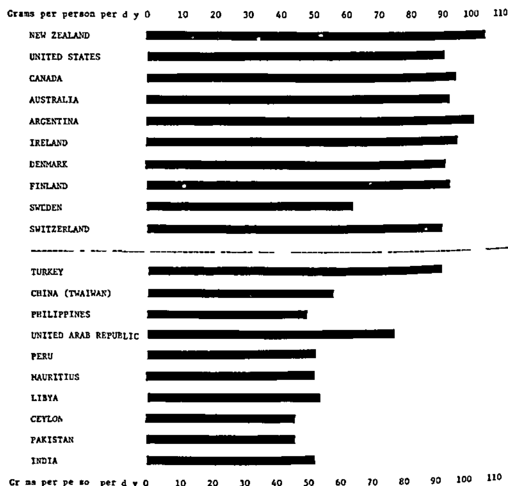


Figure 2 Available edible animal protein supplies

as the daily dietary allowances by the Food and Nutrition Board (*6*), which are 70 grams for men and 58 grams for women, with additional allowances during pregnancy and lactation In the case of Sweden, the lower total is due to lesser supplies of vegetable protein At least two (Turkey and the United Arab Republic) have a volume of total protein which will supply sufficient amounts, provided it is nutritionally adequate

It is evident from these data that there are tremendous differences in the amount of animal protein and of total protein available per person in these selected 20 countries The question before us is whether these animal protein supplies can be increased in the countries which are now low in their supply of animal protein and whether this is an economically possible or desirable thing to do Altschul (*2*) has emphasized the inefficient use of great land areas and also the opportunity for improvement in efficiency of animal production

As has been pointed out by Paddock (*8*), one of the fundamentals in advancement in underdeveloped countries is a determination of natural resources of that country and a concentrated effort to develop those natural resources We are accepting as fact the thesis that the development of animal populations can be a natural resource in most of the countries that we are considering

Beef

Beef animals fall within this category most frequently because they can be used as beasts of burden as well as to produce milk and meat It is not unusual to require from 3½ to 6 years to produce a beef animal weighing 300 to 350 kg and to require from 16 to 20 kg of feed to produce each kilogram of live-weight animal

When such is the case, the feed utilized to produce this animal is not material that is suitable for human consumption It consists primarily of heavy forage grasses and any other forage the animal may be able to find If we contrast this with the production of beef animals weighing 45 to 500 kg in one year with the requirement of not more than 8 to 9 kg of feed to produce each kilogram of live weight animal, we can see the tremendous opportunity for improvement

Part of this 8 to 9 kg of feed is made up of feedstuffs that can be suitable for human consumption However, much of the difference in time required may be accounted for by the periods of extremely low nutrition of these animals such as occurs during extreme dry seasons This may be reduced considerably by supplementing the forage diet of these animals during such periods of poor feed supplies And this can be done, in part at least by storing up feeds during the lush seasons and having it available for the animal during the dry seasons in a dry form and also by supplementing the low protein roughages or grains with either protein supplement or non-

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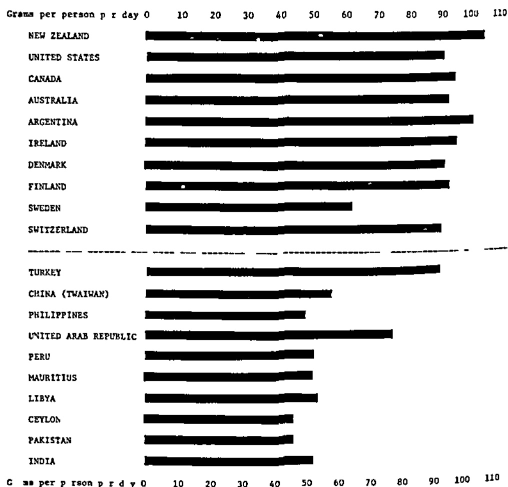


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Pigs

Another class of animal which is used to provide tremendous amounts of animal protein is, of course, the pig. Here again there are very decided opportunities to improve efficiencies in meat production from this source. Here also we have an animal that is competing somewhat with the human for the source of food. This animal requires a concentrated rather than a bulky type of feed and as such is competing for grains as a source of energy. The sources of protein are usually obtained primarily from oil seeds utilizing inedible (by humans) as well as edible supplies, but in addition, meat scraps, tankage, and inedible fish meal provide very important sources of protein for this class of animals. This animal also scavenges food waste resulting from the preparation of foods for human use.

It is evident then that there can be great improvements in efficiencies in producing animals as protein sources from the standpoint of both time, which would greatly increase numbers, and also more efficient utilization of the input of feed. Additionally, when we consider the fact that ruminants can make efficient use of nonprotein nitrogen in forming proteins

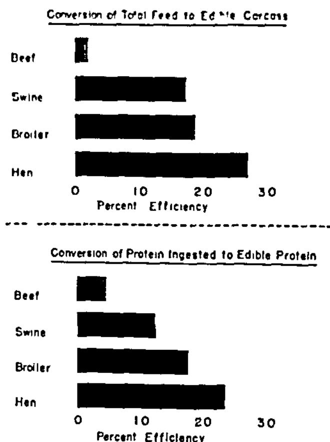


Figure 3 Conversion of ingested feed to food

Some Selected Food Sources

pe 10 (N)

Whole Egg (1)	Wheat (1)	Corn (1)	Rice (1)	Soybean Meal (1)	Cotton Seed Flour (1)
—	—	7 0	5 20	—	—
6 49	4 43	4 50	7 20	7 00	11 30
—	—	2 00	1 50	—	—
3 85	0 68	3 10	3 00	1 10	1 61
5 71	3 83	5 00	5 00	4 50	5 20
3 03	3 66	6 00	5 70	3 20	—
8 75	6 20	15 00	8 20	7 60	6 23
5 67	4 68	6 40	5 20	5 80	3 91
7 24	3 06	2 20	3 20	6 60	4 16
5 29	2 81	3 70	3 50	3 90	3 36
8 79	4 00	5 30	6 20	5 20	4 81
1 31	1 26	0 60	1 30	1 20	1 55

but primarily from unpublished data (10) The laying hen, given credit for the carcass as well as the egg produced is the most efficient of this group in converting feed to food from the standpoint of both total feed ingested and conversion of protein to edible protein The protein in the ration may be partly from inedible sources such as inedible fish meals and oil seed depending upon the area The broiler or young meat chicken, ranks second in efficiency while swine using rations of similar type, rank above beef cattle in converting total feed consumed but approximately the same in conversion of protein (12 5 vs 12 6%) The very poor performance of the

Selected Meat Cuts (Raw)

Pantothenic Acid—Mg /100 G	Biotin μ G /100 G	Choline Mg /100 G	B_{12} μ G /100 G	Folic Acid, Mg /100 G
—	—	—	—	0 013
1 0	4 6	68	2 0	0 26
0 72	5 3	120	0 9	0 009
2 0	5 5	77	—	0 007
0 59	5 9	84	2 5	0 009
—	—	—	—	—
6 40(13)	—	—	—	—
2 70	22 5	532 0	28 2	0 009
1 45	—	274 3	—	0 07
1 78	—	286 2	—	0 09

beef animal is mitigated by the fact that the material converted is, in part, very low in nutrient value, and definitely not edible for humans Conversion of calories in the feed to calories available to the human is likewise of a very low order, but the figures available are much less definitive than those for protein conversion

However, the beef carcass with 22% fat has an average of 995 calories per pound, the pork carcass with 42% fat has 1827 calories per pound, and chicken fryers with 3 33% fat have only 382 calories per pound The laying hen carcass with 14 4% fat has 784 calories per pound, and eggs with 10 35% fat have 658 calories per pound *(11)* These animals do not contribute calories in proportion to their protein efficiencies, but the contribution in this area is significant and must not be ignored

These figures illustrate dramatically the well known fact that the production of animals results in much less total food supply where animals are consuming foods that might be used for human consumption However, lack of precise figures on composition of carcasses, variability in supplementary foods, dietary habits, and other considerations, prevents valid comparisons of the nutritional value to the human of the food eaten by animals and that provided by the animal This food will vary in different areas and may be principally wheat, corn, or rice, and as available, beans, legumes, or other higher protein sources

Amino Acids

The amino acid content of selected portions of the carcass of these animals shows marked contrasts as compared with some of the more common vegetable and cereal proteins that are in use (Table I) The lysine content of the meats is noticeably higher and may therefore be used to correct dietary deficiencies Egg protein is considered one of the most complete proteins for human use, followed very closely by milk

In general, all of these animal proteins are more nearly complete in their amino acid pattern as required by the human than are the usual vegetable or cereal proteins, particularly those from the cereal grains which provide a very large portion of the protein intake for people in many countries It is true that the amino acid deficiencies of these cereal proteins can be corrected by adding amino acids But from a practical standpoint, there are still the problems of, first, the education of the average consumer to know the specific amino acids that are deficient and the means of correcting them, secondly, the quantity of amino acids to be added without creating an imbalance in the resulting product, third, cost of supplementing with synthetic amino acids or with natural food sources which are high in the specific amino acids needed, and, fourth, the availability of the necessary sources of amino acids to correct these deficiencies In other words, for some time to come, it is going to be necessary to find the most complete

diet po ible if we re to avoid spec fi deficiencies This means that there is a pl e for m a pro eins in the di t

In Table II w present sele ed vitam n analy is of on e of the more comme i meat Thi s a plu f e o and here again we find in tances where meat are the primary so rces of certain vi amins This is particularly true of B 12 and th animal pro eins are important sources of many of the others such thiam i niacin and pantothenic acid

Conclusions

The controver ial ubject of animal fats will not be argued here Suffice it to sy that fats are a good source of energy and that inevitably animal protein are accompanied by a certain quantity of fat which adds greatly to the energy supply of the world

Since time immemorial man has looked to animals to supply a very substantial part of hi food He ha al o learned to make better and more intelligent u of certain vegetable sources of pro ein such as soybeans cotton eed and peanut and we will expect the e products to be much more u eful as new and more de irable methods of preparation are de vised However it has been the hi tory of the human race that as the economic level of the population improve the quantity of meat milk and egg included in the diet increase

We are looking forward to an improvement in the economic status of all countries of the world and a we approach this goal we expect that the hum in race will not only continue to use animal proteins as a substantial part of its food but will use increased amounts in proportion to the economic status Certainly we are all looking fo ward to attaining this goal

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Conclusions

The controversial subject of animal fats will not be argued here Suffice it to say that fat are a good source of energy and that inevitably animal protein are accompa ied by a certain quantity of fat which adds greatly to the energy supply of th world

Since time immemorial man ha looked to animals to supply a very substantial part of hi food He has al o learned to make better and more intelligent u e of certain vegetable ources of protein such as soybeans, cotton eed and peanut and we will expect these products to be much more u eful a new and more desirable methods of preparation are devised However it ha been the hi tory of the human race that as the economic level of the populati n improves the quantity of meat milk, and eggs included in the diet increase

We are looking forward to an improvement in the economic status of all countries of the world and a we approach this goal we expect that the human race will not only continue to use animal proteins as a substantial part of its food but will use incre sed amounts in proportion to the economic status Certainly we are all looking fo ward to attaining this goal

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General Outlook For Milk Proteins

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Milk protein holds great promise for the future nutrition of man. Unfortunately, three-fourths of today's milk is produced in Europe, North America and Oceania with only one-fourth of world's population. Milk production is possible in all developing nations and concerted efforts are being made to establish milk industries throughout the world. Increasing population pressure questions our ability to support animal agriculture which competes for the plant protein supply. Ruminants by their unique microbiological digestive process are capable of utilizing crude forms of protein, free nitrogen and synthesizing animal protein; the cow can produce normal milk protein without protein in her diet. Our reservoir of scientific resources should permit milk protein to remain in our scheme of food production for future generations.

As we analyze the present and view the future prospects for milk proteins in the human diet, we must take a very hard look at the problem in all its aspects. From the beginning of recorded history we find evidence that milk and other animal protein foods have played an important role in man's nutrition and development. The strong and stable nations and cultures have all been developed and maintained with the help of a system of animal agriculture. The ruminant, particularly the dairy cow, has played an important role in this picture through her unique ability to convert crude forms of forage and grains into milk, a high quality protein food for man.

As the human population explodes at an alarming rate, the prospect of man's having to compete with animals for the products of the soil looms larger. We are more frequently being confronted by the question "How long will we be able to afford the luxury of utilizing our lands for forage and grain production to produce the highly refined animal protein foods?" Domestic animals are admittedly relatively inefficient in their conversion of feed to human foods. The dairy cow converts only 23% of the protein

she consumes into milk and meat, the beef animal only 10%, and the pig 12%

These facts viewed in the light of current food production trends throughout the world add up to a rather dismal picture One billion of the 3 billion humans on the earth are already underfed or malnourished, and prospects for rapid improvement are not very bright A recent report by the USDA Economic Research Service (4) suggests that the less-developed countries of the world are clearly losing the capacity to feed themselves and that a growing share of the increase in population is being sustained by food shipments from the developed countries The report attributes this situation to the fact that the densely populated, less-developed countries have nearly exhausted the supply of new land that can be brought under cultivation Since nearly half of the world's population lives in these less-developed countries, the problem cannot be passed over lightly

Irrespective of what has been or will be said about the merits or potential of the protein products of the sea, the plant proteins, the seed proteins, or the synthetic protein foods of the future, the vast majority of humans are not yet prepared to give up the foods that come from an animal agriculture There are many hopeful signs on the horizon, and I feel confident that with the scientific ingenuity at our disposal and with the many national and international agencies now actively attacking these problems, solutions will be found

Present Status of Milk Production

Many of the less-developed and emerging nations of the world are in dire need of animal proteins At the present time the populations of the Far East obtain only 6% of their caloric intake from animal products Those living in Africa average 7%, while those inhabiting the Near East get 8% of their calories from this source (5) When these are compared with the diet of the North American, who derives 35% of his calories from animal products, the problem begins to come into focus This disparity exists in spite of the fact that most of the countries in the low animal-calorie regions are basically agricultural and frequently possess large livestock holdings In Kenya, East Africa for example a country of 8,600,000 inhabitants, there are a cattle population of nearly 7 million, about a quarter of a million camels, and 13 million sheep and goats Most of the cattle are kept for reasons of prestige in the community and with complete disregard for quality and economic considerations As veterinary services have improved and disease control practices have been adopted, the number of these unproductive cattle has increased, bringing further deterioration of pastures and soil It has been reported that at least 20 milking cows are required to supply the milk needed by one nomadic farm

family (12) The calf takes precedence over the needs of man so a yield of 200 ml to 2 liters of milk per day is about all that can be expected from these native cattle during their short lactation periods

India a predominantly agricultural country with a population of 440 million people has a cattle population of 220 million Admittedly a substantial number of these cattle used for draft purposes and other non milk producers are kept because of religious beliefs and customs which prohibit their slaughter Approximately 48.5 billion pounds of milk are produced in India per year about 5 ounces per person per day However, 60% of this milk is converted into milk by-products particularly ghee the manufacture of which results in an almost total loss of the milk protein (9)

Conversely in the developed nations of Europe North America and Oceania milk production and the milk processing industries have developed dramatically over the past 25 years On these three continents where only one fourth of the world population lives three fourths of the world's milk is produced (14) From USDA reports we find that in 1945 the United States had 27.7 million dairy cows that produced slightly less than 120 billion pounds of milk Today we have 10 million fewer cows (17.6 million) producing nearly 127 billion pounds of milk The average production per cow per year has risen 2000 pounds in the past 10 years to a present level of 7880 pounds The cows in herds under the Dairy Herd Improvement programs (1.7 million cows) averaged 11,685 pounds last year and are still increasing at a uniform rate of about 2% per year This has been possible through genetic improvement greatly improved methods of feeding and management and the development of markets and economically sound marketing methods In North America we consume about 1 quart of milk (in all its forms) per capita per day while the average Asian is limited to about 1 quart equivalent every 14 days (5)

The milk production potential of the United States has never been tested We are producing adequate amounts to meet the needs of our people with a 5 to 7 billion pound surplus annually Our efficiency of production improves each year and more milk is being produced per acre than ever before This trend promises to continue for some time to come The current annual production per cow of 7880 pounds in the United States is far below the potential Many well managed herds now average 15,000 to 16,000 pounds per cow per year and it is predicted that the national average could reach that level in the foreseeable future Many other developed countries have the land, climate, cattle and technical capability to attain much higher levels of milk production if the demand arises I feel confident that the developed nations of the world will be able to afford the luxury of milk and its products in the foreseeable future and still supply a limited amount of milk protein foods to their less fortunate brothers in the less-developed and emerging nations

The Problem

Possibly no group now working in the international field is better informed on this problem and its many ramifications than the Animal Production and Health Division of the United Nations Food and Agri cultural Organization The chief of the Dairy Branch of the FAO, Hans Pedersen and his associates have analyzed this problem in the light of the predicted doubling of the world's population by the year 2000 (*14*, *15*) They contend, through experience in many countries, that milk production is possible in any country of the world but that production alone without a system of processing and distribution is futile Native farmers who respond to programs of genetic improvement and forage production are frequently discouraged by the fact that they have no nearby market for their surplus milk over family needs When they do find a market it is often one for cream, in which case they must feed the skim milk to their cattle or discard it FAO contends that the need in the world is not for butterfat but for the milk protein in the skim milk Some very substantial progress has been made on this particular problem Through FAO and UNICEF cooperation and help several rural cooperative milk processing centers have been developed in Kenya These diesel-powered plants, which were built in very remote areas of this agricultural country, started by receiving as little as 12 gallons of milk per day (2 gallons per farmer), but now they process over 1000 gallons a day each (*12*, *13*) These plants have provided a market for the milk produced and thereby stimulated further production by the native farmers Contrary to common belief, it has been demonstrated that the climates of the tropics and subtropics are not serious obstacles to milk production by highly productive dairy cattle Israel and other hot countries have introduced environmental modifications which make it possible for European and American breeds to produce milk at levels comparable to that in their countries of origin (*10*)

Processing Facilities

Assuming that efficient milk production could be established in all of the less-developed countries of the world, a very substantial problem of plant processing facilities would exist A very recent analysis by the FAO Dairy Branch (*14*, *15*) is based on the fact that one billion people in the world are underfed or malnourished With this existing base and with the assumption that the world population is increasing at a rate of 50 millions per year, they have made some projections as to the need for new dairy processing plants over the next 10 years Assuming that the present 1 billion underfed were to receive only 0 25 liter (0 26 U S quart) of milk equivalent per day, there would be an immediate need for 2500 new processing plants each with a capacity of 100,000 liters per day In

addition to provide milk at the same rate for the anticipated increase in population over the next ten years would require 125 more new plants of the same size each year. Put together this means that by 1976 3650 new plants of 100,000-liter capacity would have to be built. In other words one new plant would have to be built, equipped and opened each day of the year for the next ten years even to catch up. Then at the end of 1976 we would have processing capacity to provide milk and milk products for the underfed and the increased population at a consumption rate only one fourth that of the current per capita utilization on the North American continent.

Outlook

The outlook for increasing milk protein resources is not a simple matter. Were it merely a clear-cut problem of production, processing, and distribution the future might lend itself to some sort of concrete prediction. Unfortunately, when one considers the problem with its many complicated sociological, political, economic, geographic, climatic, religious and biological variables intertwined it becomes a far greater problem than a mere dairy and food scientist can cope with. Of one thing we can be sure, progress will be slow and difficult. A specific example should demonstrate this point. One essential step in promoting dairy industry development throughout the world was the establishment of a world wide code of principles covering standards of identity and analytical methods for milk and milk products. Such a code, now adopted by 58 countries, required nearly 10 years of intensive work by the International Dairy Federation, the World Health Organization and FAO (7).

There are, however, a few obvious conclusions that one can draw from history and the experience of those who have been struggling with this problem for a number of years. There are also some untested possibilities that can and should be considered for the future.

At the outset it is obvious that charity, the shipment of nonfat dry milk or milk in other forms to needy nations, is not the permanent solution to the problem. Charity temporarily alleviates starvation in periods of famine and will cure infants and children of the protein-deficiency disease kwashiorkor, but it does not begin to provide the basic and continuing needs of the population as a whole.

Likewise the problem cannot be solved by much talking and quoting of statistics. Up to a point this defines the problem and helps develop methods for solution but sooner or later action programs must be initiated.

An oft-quoted ancient Chinese proverb may well be the key to the problem of providing a more adequate supply of milk protein around the world. "Give a man a fish and he will eat for a day. Teach him to fish and he will eat for the rest of his life." Education is one of the essential

ingredients needed to solve the food problems of the world. Not education as we view it in the United States, where the bachelor's, master's, or doctor's degrees are the goal of millions, but education in the broadest sense, which starts with enlightening the masses at the most elementary level. The heart of the problem starts with the fact that illiteracy and hunger are usually found together. A hungry man cannot work efficiently and thereby develop purchasing power to acquire the food he needs. The internal social and economic status of a country must be conducive to developing the market for milk and other quality foods before production of these foods within that country can be undertaken successfully.

At the level of milk production, education again must play an important role. It is generally recognized that the dairy industry in the less-developed nations must literally emerge from the grass roots and that the mere acquisition of a knowledge of the technology of cattle management, feed, and pasture production is not enough. This knowledge must be conveyed to the peasant. In most of these countries, where the majority of cattle owners are illiterate and the more enlightened have little more than 4 to 5 years of schooling, the problem becomes a real challenge (*2*). Programs are now emerging in which formal and informal training is being accomplished through such elementary techniques as word of mouth, reading, memorizing, watching, and doing under supervision. It is a monumental task but a necessary one.

Once the markets, the initiative to produce, and the system of distribution are developed, the plane of nutrition should rise and give further impetus to social and economic progress.

No one is so idealistic as to believe that the deficiency of milk protein in the world can be corrected overnight. The best laid plans and goals must be keyed to other related economic, social, and political factors which must evolve through an evolutionary rather than a revolutionary process. The philosophy of FAO on this point is well expressed by the following quotation (*14*):

Don't try to solve all problems at one go! If you cannot get a pint of milk a day, then get a pint every other day or at least every week. Do whatever you can with the available resources—but do it now!

For the present, therefore, we should be reconciled to the fact that those nations that "have" will need to continue to help provide the milk protein for those that "have not."

This help, both by donation and through normal trade channels, will of necessity be confined largely to nonfat dry milk, which normally contains about 36% milk protein. This product is considered the most valuable source of complementary protein for the malnourished and lends itself to distribution and storage conditions prevalent throughout the world (*11, 20*). A program has recently been adopted wherein all nonfat dry milk moving

overseas under the U S Public Law 480 program will be fortified with vitamins A and D *(20)* This product properly reconstituted in potable water will at least meet the minimum needs for infants children, and mothers who might otherwise get little or no animal protein in their diets For countries with limited milk supplies and for those which will develop modest milk supplies in the future dry milk can be used for ' toning, a practice of blending local high fat milk supplies with reconstituted nonfat dry milk *(6 14)* This method of dry milk utilization appears to hold much promise for augmenting the milk protein supplies of countries struggling to develop and expand their own system of milk production At about 23 cents per pound of milk protein nonfat dry milk is one of the cheapest forms of animal protein available on the world market (6) For this reason it is particularly well suited to the developing countries which are seeking practical solutions to their animal protein problems In 1964 the United States produced 2 2 billion pounds of food grade nonfat dry milk and we have the capability to produce much more

The ultimate goal however should be to develop dairy industries in all countries of the world and wherever possible they should be founded predominantly on local milk production Then and only then can a population be assured of a continuous and adequate supply of milk protein

A Bold Look Ahead

We have seen that by expanding the use of our existing scientific knowledge disseminating the technology of the more developed countries, and sharing our own resources we can help meet the growing need for milk protein for many years in the future However we have still not answered the question Will the day come when we must begin to think of abandoning our animal agriculture so that released land can be used for more direct and intensive production of human food? The passing of feed materials through animals is not the most efficient means of human food production, although the quality and palatability of the ultimate product are greatly enhanced It may not be too early to consider some bold and radical alternatives to our conventional method of producing milk protein Let's examine one possibility

Ruminant animals—cattle sheep, and goats—may offer us a unique opportunity to maintain an animal agriculture almost indefinitely These animals are all characterized by having a four part stomach, the first of which is the rumen This organ does not possess animal enzymes but, rather contains billions and billions of bacteria and protozoa These microorganisms actually bring about the digestion of the feed materials which the animals eat The cow or sheep thereby derives its nutrients by absorbing the soluble products of the microbial fermentations or by actually digesting the microorganisms in the lower digestive tract

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We already know that the rumen microorganisms synthesize the B vitamins and the essential amino acids which are secreted in milk. The question we wish to raise is, "Can we extend the usefulness of the rumen microorganisms beyond what we now require of them?" By this we mean, "Is it possible to have these organisms synthesize more and more of the essential nutrients a cow requires so that they need not be provided in the animal's diet?"

The pioneering studies of A. I. Virtanen, Nobel Laureate from Finland, indicate that this may indeed be feasible (*17*, *18*, *19*). Virtanen has been working with highly purified diets for milk production by the bovine species. These diets are composed of purified starch, sucrose, α-cellulose, urea, a mere trace of corn oil, ammonium sulfate, ammonium phosphate, 16 other minerals, and vitamins A and D.

The dairy cows fed this ration over extended periods of time have maintained normal body functions, body weight, and reproduction. Milk production has been maintained at about half the level of normal high producers, or about 4500 pounds per year. The major point of interest here is that this milk is completely normal in every respect—its content of proteins, vitamins, and the other normal chemical constituents is the same as that of milk from animals fed normal, complete diets. This observation has far-reaching implications, because it demonstrates that the ruminant is potentially capable of producing milk protein without competing for the available plant protein supply. In this study, high quality animal protein food was produced without feeding the animal any protein whatsoever. Virtanen states: "If a cheap, sufficiently digestible carbohydrate feed can be prepared from straw, wood, sugar cane waste, or other fast-growing plants of the same kind, or even synthetically, it may be possible to remove, by milk production, protein and vitamin deficiency in vast areas inhabited by undernourished peoples" (*18*).

An example of a useful raw material of this nature is the 15 billion pounds of whey that is available each year as a by-product of the cheese industry in the United States. At present whey poses a serious disposal problem. A process has been developed in our laboratories at The Pennsylvania State University for the production of a high nitrogen cattle feed from this whey by fermentation with *Lactobacillus bulgaricus*, followed by treatment with anhydrous ammonia to neutralize the lactic acid formed (*1*). Animals performed satisfactorily when this material was included in their ration as a source of nitrogen, although problems of palatability were encountered and are yet to be solved (*8*).

Research should begin really to test the potential of rumen microorganisms to support animals when they are receiving an odd variety of materials which have little or no food value to man or do not compete with land used for human food production. This work should also extend into studying what chemical or microbiological processes might transform

substances which we do not now consider feed materials, into highly desirable ingredients for the ration of the ruminant Likewise, extensive studies on ways of improving the palatability of rations are needed The classic and continuing studies of Virtanen have already shown that one amino acid histidine, is apparently the most limiting factor in purified diets This suggests that supplementation with this one amino acid might greatly increase the production of milk protein in animals receiving the purified or exotic diet Now is the time for this type of information to be obtained so that when and if conditions require a modified animal agriculture, man will be prepared to maintain his plane of nutrition without drastic changes in his diet

Summary

This discussion on the outlook for milk proteins has dealt primarily with the problems of the less-developed countries of the world because it is in these countries where the problems exist The developed nations have adequate supplies and even surpluses now and will develop the capabilities needed to continue animal agriculture in their schemes of food production for the generations ahead We should nevertheless be prepared to apply alternative methods of animal protein production which will not compete with supplies of plant protein needed for direct human consumption

The immediate goal should be that of helping the milk-deficient nations develop independent milk production, processing and distribution systems which with some augmentation through imports of nonfat dry milk will help elevate their plane of nutrition This promises to be a long tedious and monumental task which will be compounded by the pressures of population growth The problem is not one that will be solved with our scientific and technological tools alone but will require an integrated effort in education and social change With many organizations within the United Nations and other international national, educational, and private agencies already actively working on these problems, and with the reservoir of scientific and technological resources at our disposal, there is certainly room for optimism

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General Outlook for Edible Fish Protein Concentrates

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Oily species are probably the most abundant potential raw material for fish protein concentrates (FPC) Without solvent extraction FPC will contain up to 10% oil, usually highly rancid Some people like rancid flavors, but there are nutritional objections The oil can be stabilized by antioxidants or by partial replacement with vegetable oil, but there are technological problems Partial removal of oil—e g , with hexane—gives FPC with some remaining flavor and negligible nutritional hazard Extraction with ethanol or 2 propanol removes all lipid but also all taste, and the FPC must be added to a staple food Flavor "reversion can be troublesome Technological, economic and sociological problems must still be overcome before FPC becomes an important commodity, but the outlook is promising

In many quarters it is being said that man must turn increasingly to the oceans for the extra food needed by the world's growing population Certainly the biggest part of the world food problem is the over all deficit of high quality protein, and food from the ocean consists in the main of such protein It is debatable, however how far present fish and other seafood supplies can be increased, using the traditional approach in which the fish stocks are exploited by hunting but are not husbanded like farm animals It may be that fish farming on a major scale will one day be accepted practice, but for the present purposes only the existing fisheries pattern is considered

The world catch of fish in 1963 was 46 4 million metric tons (*12*) About one quarter of this was not caught for the human food market but for conversion into animal feedstuffs This is a measure of the extent to which current supplies could yield additional food for direct human consumption It is worthwhile to consider briefly the pattern of the world

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80°F (27°C) when packed in a hermetically sealed container (*11*) Fish meal is undoubtedly the cheapest potential fish protein concentrate, but is not intended for human consumption Just how much cheaper it is than the more sophisticated products is illustrated by some data cited by Abbott (*1*) Anchovy meal f o b Lima cost 10 5 U S cents per kg, with a protein content of 65%, compared with a deodorized, defatted Moroccan FPC, with 80% protein at 40 cents per kg It is essential that FPC should be as cheap as possible, because of the poverty of its potential consumers Is there any reason why ordinary fish meal should not be fed to protein deficient populations, even to babies and young children?

First fish meal is not normally made under sufficiently hygienic conditions to rule out the risk of occasional contamination by pathogenic bacteria No conventional fish meal plant is so designed that all pockets of bacterial reinfection can be eliminated In any case, it is not acceptable practice to produce human food and animal feed in the same plant Completely separate premises, plant, and personnel would be needed, which could put up the price a little

Second, although fish meal has given satisfactory results in animal rations, the animals in question are living on very carefully balanced diets, with adequate amounts of such things as fat-soluble vitamins Malnourished humans generally suffer from multiple deficiencies Fish meal typically contains rancid fat which could conceivably precipitate a deficiency of fat-soluble vitamins There are, however, a very few and quantitatively unimportant fish meals that do not become rancid

Third, there is always a slight risk of cumulative toxic effects when a product that is not proved to be wholesome is fed over a very long period Farm animals do not live out their full lifespan, often their life is extremely brief Under a thoroughgoing scheme, humans would probably eat FPC every day from weaning to old age Apart from its destructive effect on fat soluble vitamins, many nutritionists look askance at highly rancid fat, such as occurs in fish meal, as a possible toxic agent

On the favorable side many protein-deficient populations actually like the flavor of really rancid fish products This does not, however, mean that the products are good for them Some time ago an experiment was made in Peru with dried, whole anchovies The Indian population in the Sierra country ate these with relish, despite their rancidity, or perhaps because of it! But illness followed, and the experiment had to be abandoned Dried oily fish have a far higher oil content than fish meal made from the same raw material so that this experience does not mean that anchovy meal would have produced the same troubles But it does suggest the desirability of keeping the level of rancid fat in FPC as low as possible White fish meal, with a crude fat content generally less than 6%, might be satisfactory, but some nutritionists would prefer to see it considerably lower and to think in terms of total fat, rather than crude fat, which means

what can be extracted with ethyl ether or light petroleum Badly oxidized and polymerized fat is not extracted by these solvents

The amount of FPC suggested for inclusion in human diets should supply about 15 grams of protein, at about 70% protein content this means about 20 grams of FPC which, if it consisted of normal oily fish meal with about 10% residual oil, would contribute some 2 grams of rancid fat It seems most unlikely that this quantity involves any significant risk and present thinking in the international agencies is that unsophisticated fish meal, if made under satisfactory processing conditions, might well be the best product for a large proportion of the world's potential FPC consumers

Some workers advocate the use of commercial fish meal as raw material for further processing into FPC, typically by solvent extraction The extensive South African research and pilot scale developments have been based on fish meal Such use of fish meal ensures continuity and even rate of supply of raw material and permits maximal recovery of the best type of oil, since oil recovered by cooking and pressing as in fish meal manufacture is in general superior to that extracted by solvent for use in hydrogenated oil and margarine, although careful operation and use of suitable solvents can give oil of high quality Solvent-extracted fish oil is more uniform and more stable than pressed oil, but where color is a factor, refining losses will be greater

There is considerable experience showing that fish meal made from very fresh raw material—e g , made at sea on factory ships—is easier to deodorize and deflavor by subsequent solvent extraction than meal made from staler raw material A Polish project now in the development stages will be based on fish meal made at sea as raw material for solvent treatment ashore (*22*) Delay between manufacture of fish meal and subsequent solvent extraction also increases the difficulty of complete removal of odor and taste, because of oxidation and polymerization of the residual oil in the meal There is much to be said for the use of freshly made press cake as raw material for subsequent solvent treatment, but continuity of supply could not be guaranteed, and no current project appears to be based on it

Two types of FPC that are really sophisticated fishmeal are envisaged by the Protein Advisory Group of the United Nations Agencies most closely involved (FAO, UNICEF, and WHO) One (type A) is virtually odorless and tasteless and has a maximum total lipid content of 0 75%, the other (type B) has a maximum total lipid content of 3%, with no specified odor and flavor limits Total lipid means material extracted in 6 hours by boiling ethanol Much of it may, in fact, be nonlipid material It is accepted that, even if rancid, the fat level in type B offers no significant nutritional hazard Inevitably, however, solvent extraction is involved in the manufacture of such products, and the tentative specifications include zero levels of "harmful" solvent residues As always, zero level specifications give trouble as analytical methods become more sensitive, but for

the e sophisticated products the tentative specifications also require safety tests with animals Apart from solvent residues, there is always the possibility of toxic reaction products of solvent and fish

There are types of FPC that are totally unlike fish meal These are typically extensively hydrolyzed fish protein that resembles meat extract rather than fish Hydrolysis may be either chemical or enzymatic, and the final products may be concentrated to a pasty consistency or incorporated into bouillon cubes An example of the enzymatic approach is that of Bertullo (*3*), employing a proteolytic yeast, while HCl hydrolysis of white fish meal pre-extracted with water was used by Stachowski (*22*) So far the hydrolyzed products are a long way behind the variants of fish meal, in both development and potential demand

Table I Analysis of Some Types of FPC

(G /100 G dry matter)

Sample[a]	*Protein* (N × 6 25)	*Fat*	*Ash*
Menhaden meal (*15*)	65	11	22
Herring meal (*15*)	76	11	11
Pilchard meal (*14*)	71	7	—
Ethanol-extracted (*8*) pilchard meal	79	0 6	19
2 Propanol extracted (*21*) cod flesh	96	0 03	5

The first three sample are normal commercial fish meals (FPC type C), the fourth is FPC type A made from whole fish the fifth FPC type A made from fillets only The e products are typical of many others Type B is intermediate in fat content it has received far le s attention Fat in the first four samples is crude (hexane) fat in the last sample it is total ($CHCl_3$/MeOH) lipid

Production Processes and Problems

It is not intended to discuss in detail all the methods that have been propo ed or developed for producing the sophisticated fish meals known as FPC types A and B A good summary of many of them has been given (*24*) Nor will any attempt be made to describe the manufacture of conventional fish meals Rather will attention be concentrated on certain general processing features with particular reference to the chemical and physical problems that are associated with them Any sophistication of conventional fish meal, whether the raw material is fresh fish, commercial fish press cake or commercial fish meal is essentially designed to remove undesired odors and flavors, or prevent undesired changes between manu facture and consumption

The odorous and flavorous components of fish fall broadly into two groups, the water soluble "extractives which are largely responsible for

fat remained constant, and the meal did not become rancid on exposure to the air. It tasted like a freshly smoked herring product, such as a kipper. As a matter of fact, it was known from wartime dehydration studies that whereas dehydrated herring rapidly became rancid, dehydrated smoked herring did not (7). Wood smoke is also known to prevent oxidation of fish oils as such (20). It is not suggested that simple smoked fish meal would be a suitable type of FPC, in view of the small but definite carcinogen content of wood smoke, but it might prove feasible to incorporate a suitable antioxidant by fine dispersion or vaporization into the dryer gases. Alternatively a carcinogen-free wood smoke might be used, since the smoked FPC flavor should be acceptable to many people.

A different line of approach towards prevention of rancidity in oily fish meal is partial replacement of the residual fish oil by a more stable type of oil, such as a vegetable oil, which furthermore contains natural antioxidants. This has been shown to be technically feasible (9) and should be effected with a freshly made fish meal. Using peanut oil for replacement, the fish meal, while still containing the typical 10% of fat, remained completely free from rancidity during at least 4 months' exposure to the atmosphere. The problem is what to do with the expressed mixture of vegetable and fish oil, which is dark in color and rancid in odor. The amount of it is approximately equal to the amount of vegetable oil originally added to the fish meal, which is then warmed and pressed—in the preliminary South African experiments about 15% by weight of the fish meal. The economics of oil replacement will depend largely on the monetary value of this recovered oil, which has not yet been determined.

Little need be said about production problems for FPC type B. Relatively cheap solvents, such as feed grade hexane, can be employed either to extract most of the fat from a dry raw material such as fish meal or to remove both water and fat from a wet raw material by a process of combined azeotropic drying and solvent extraction. The solvent with which most experience has been gained in the latter type of operation is 1,2-dichloroethane (5, 16). Manufacture of FPC type B is facilitated and cheapened by the fact that the solvent extraction need not be exhaustive. The problem of residual solvent traces is dealt with under FPC type A, but it is potentially worse for type B because the solvents in question are probably more toxic or at least suspect.

The cheapest and safest solvent for extraction of a food product is obviously water. Starting with a low-fat fish meal such as cod meal, water extraction will remove not only the water soluble extractives but at least part of the phospholipids which in such meals form a considerable proportion of the total lipids. The final product could in many cases be regarded as FPC type B, but its flavor is entirely different from that of fish meal extracted with such a solvent as hexane. For certain purposes it is superior —e g , as raw material for further processing into a bouillon like seasoning

(*22*) It can also be incorporated into bread or biscuits at a higher acceptable level than can the original low fat fish meal (*23*)

If a manufacturer is producing simultaneously FPC of all three types (A, B, and C) for different markets, it may well be cheapest for him to make type B by mixing types A and C Provided the flavor and texture are acceptable, there seems no obvious objection to this

The really tough technological problem is provided by FPC type A Practically the total lipid of all types and the total water soluble extractives must be removed Solvent residues should be below the level of detection by taste or smell There must be absence of substances capable of producing taste or smell as result of storage changes When a product is virtually odorless and tasteless, sensitivity to any odor and flavor still in it is enhanced An example is furnished by what happens if acetone is used as a solvent at any stage There have been several commercial proposals to use acetone to extract both water and fat, and the economics of the process, including solvent recovery, are attractive Acetone, however, invariably leaves the final product contaminated with traces of strongly flavored condensation compounds, which cannot be removed below the level of taste detection even by exhaustive subsequent extraction with other solvents, or by any normal desolventizing procedure It is possible to get a product with no perceptible odor of acetone condensation compounds, but they will still be repulsively obvious to the sense of taste

The solvents which have been successfully used to make FPC type A are the lower aliphatic alcohols methanol, ethanol, or 2 propanol They can be used to extract water water soluble extractives, and lipids direct from fish or fish press cake, to remove both water soluble extractives and lipids from fish meal, or for a second stage extraction of FPC type B Methanol is such a poor fat solvent that it has really proved valuable only in this last type of operation where the residual lipids are mainly phospholipids Apart from azeotropic drying, the only advantage in using a two-stage process, such as hexane extraction followed by ethanol extraction, is in respect to solvent costs, particularly where excise problems are involved Since 2 propanol is free of excise tax, it has tended to replace ethanol—e g , in Morocco (*13*)

The difficulty of finding a solvent with comparably good extracting powers for the entire range of flavorous and odorous components of fish has led to the use of mixed solvents Typically some water is added to a solvent such as ethanol or 2-propanol If the raw material is fresh fish or press cake, account is taken of the original water content Thus the multistage process developed at Halifax (*21*) using cod flesh as raw material involves first addition of 99% 2 propanol to give a final 70 to 30 2 propanol-water mixture, then further extraction with 70 to 30 2 propanol-water, followed by extraction with 99% 2 propanol With oily raw material addition of too much water will hinder fat extraction, thus the Moroccan process (*13*)

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employs a mixture of 80 to 90% 2-propanol, 10 to 15% hexane, and 2 to 3% ethyl acetate to extract sardine meal with some 6% initial water content. Much work has been done in South Africa on the extraction of oily fish meal with ethanol. Tests (*18*) with various ratios of ethanol to water, ranging from 100:0 to 25:75, showed the optimum to be about 90:10.

It is unlikely that any particular solvent mixture or extraction sequence will be the best for varying types of raw material, and a good deal more work is necessary in this field. Many samples of FPC which immediately after manufacture are claimed to be odorless and tasteless are neither when submitted for comments some time later. This well-known tendency to "revert," whereby a perceptibly fish meal–like odor and taste develop, indicates incomplete extraction of some precursor or precursors. Certainly the products are not entirely free from either lipids or water-soluble substances. Thus the Canadian product still contained about 0.02 to 0.04% of lipids extractable by chloroform-methanol (*21*), while the best product in the South African experiments (*18*) contained over 1% of material recoverable by further exhaustive hot alcohol extraction. This material was probably largely water-soluble and nonlipid, and despite its presence it is claimed that such products do not show flavor reversion (*10*). The FAO tentative specifications (*11*) allow up to 0.75% of material extractable in 6 hours by boiling ethanol. Current researches in the United States will, it is hoped, lead to a fuller understanding of the chemistry of flavor reversion in FPC type A (*24*).

One practical problem in the manufacture of FPC type A is most likely to be appreciated when actual production is going on, rather than research. Some of the extracted compounds are volatile and contaminate the recovered solvent. In so far as the volatile contaminants are basic or acidic, they can be removed chemically, but South African experience with ethanol recovery has been that the only really effective procedure is charcoal purification in the vapor phase during distillation (*8*).

Extremely little work seems to have been done on the possibility of extracting the protein from fish meal, leaving behind the bone, and subsequently purifying the extracted material. Preliminary experiments at Torry Research Station (*6*) have shown that much of the protein of commercial fish meal can be extracted with anhydrous formic acid, and that when the extracts are mixed with ethyl ether the protein is precipitated and most of the contaminants are left in solution. Washing the precipitate with ether leaves an odorless and tasteless product. This very preliminary work is mentioned only to show that possibilities exist other than the line generally pursued in preparing FPC type A.

At the present stage of research and development it does not seem profitable to discuss the processes and problems of making hydrolyzed types of FPC.

Application and Use

Despite great efforts by the United Nations special agencies (particularly FAO and UNICEF), individuals and manufacturers, FPC has not yet found a continuing role in combating malnutrition in any country. The problems of its manufacture are less recalcitrant than those of its widespread introduction in the areas where the need is greatest. In part the problems are economic: a suitable product is not available because the market is not established and the potential manufacturer cannot see where his reasonable profit is to come from. In part the problems are rooted in conservative food habits and dietary patterns. In part the problems arise from ignorance of nutrition on the part of the great mass of the people involved and from the common experience that people will not buy and eat something solely "because it is good for them."

FPC type C—namely fish meal prepared under satisfactory conditions for human food—is directly acceptable as a tasty item in large areas of the world. Thus in Malaya it was given dry by spoon straight from the tin, to both mothers and children (25). The usual immediate comment of the mothers was that it was very good and like the small dried salted fish already familiar in their diet. It was also acceptable to all racial groups when added to cooked rice or to vegetable soup. Experience in other areas has been generally similar. In such cases the marketing problems are likely to be simplified but there is a potential problem with possible excessive intake as discussed above. There is far less inherent risk of producing new nutritional imbalances if the FPC is incorporated at a reasonable level in some staple food. This will almost certainly be necessary with FPC type A, the other main contender in most current proposals. Admixture of freshly dried fish or press cake with vegetable products containing natural antioxidants may incidentally prove a good way of preventing excessive rancidity in oily FPC. This is the basic idea behind one of the research contracts of the U.S. Bureau of Commercial Fisheries (24)—*viz.* work on mixtures of dried fish and "tempeh," a fermented soybean preparation popular in Indonesia. If rancidity is not serious, the oil content of FPC type C is a distinct nutritional asset to populations short of both protein and calories.

Incorporation of a tasteless FPC into a staple food, such as bread, will demand government support and either a subsidy or compulsion directed towards the industry making the staple food in question. The subsidy approach was tried in South Africa, but the wrong medium (brown bread) was chosen for enrichment. The wrong population groups received the FPC and the experiment was abandoned. However, South Africa is to be congratulated on having now developed the only existing commercially successful outlet for FPC type A, the cereal type breakfast food, Pronutro. The full production capacity of the South African FPC pilot plant is

absorbed by this product which, although it does not reach the areas of greatest need, does keep alive and healthy the production facilities for FPC, an important part of the general problem of getting the product into use

The need for further market research in respect of FPC is often emphasized, but enough has already been done in some areas to get a few practical schemes into operation Inertia at various levels can be a greater problem than production and acceptability

Conclusions

A large part of the world's fish catch, at present converted into fish meal for animal feed, could with advantage be converted into FPC for direct human use Additional fishing effort could also contribute to this end Basically most types of FPC resemble fish meal in being dried, comminuted fish tissues, with most of the oil removed from oil rich raw material, but for use as human food handling and processing must be carried out under conditions of hygiene impracticable in the existing fish meal industry Two types of FPC appear to be most important in present planning One type contains all the ingredients, including the oil, present in fish meal It has a strong flavor liked by many potential consumers, but on nutritional grounds it is desirable to limit the extent of rancidity in the oil fraction This should be possible by suitable use of antioxidants, although more research is necessary on their mode of incorporation The other main type of FPC is a highly sophisticated product resembling a fish meal from which nearly all the oil and nearly all the water soluble material have been extracted by suitable solvents, leaving essentially only protein (and bone if present in the raw material) It must be virtually odorless and tasteless and must remain so on storage The last requirement in particular is one on which further research is needed This type of FPC, while nutritionally an admirable protein supplement, in contrast to the first type has no appetite appeal and can probably find a large scale use in the areas of the greatest need only with the active and continuing support of the governments concerned

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Discussion

D G Snyder I should like to present information on two items concerning FPC that Dr Lovern did not cover in his excellent paper—yet these items I feel are critical to matters being discussed at this symposium We should first of all have some idea about how much fish is potentially available for FPC manufacture from our world's oceans on an annual sustained basis

The next obvious point is to indicate how much this high quality 80 to 85% animal protein product prepared from whole fish is going to cost

There are almost as many estimates of potential fish harvests as there are biologists making these estimates. Let us consider a high, medium, and low estimate.

Wilbert Chapman of the Van Camp Foundation estimates, and provides the calculations to go with his estimate, that the total annual production by the ocean of fish and shellfish of practical usable size is 2 billion tons. This tonnage would supply, in turn, 400 million tons per year of animal protein. This tonnage of protein would, of course, be adequate to meet the annual requirements of a world population ten times the size of the present one.

Bureau biologists estimate that about 500 billion pounds of fish could be harvested annually from our world oceans. Let's assume (and these are my calculations) that from 500 billion pounds of fish we can get roughly 100 billion pounds of FPC. If we consider that 10 grams comprise one supplemental dose of animal protein and 400 days comprise one year (the former value may be just as wrong as the latter one), we could provide 10 billion people with an animal protein supplement each year from now on, assuming all fish harvest would go into FPC production—which it would not. Today about 100 billion pounds of fish are taken and used for purposes other than FPC. Therefore, only four fifths of the potential would be available for FPC production. Instead of 10 billion people, we supply FPC supplements to only 8 billion people.

Herbert W. Graham and Robert L. Edwards, Biological Laboratory, Bureau of Commercial Fisheries, Woods Hole, Mass., have provided one of the most conservative estimates of the annual harvest potential of our oceans. They conclude that under present conditions and with present fishing techniques, the annual harvest of marine fish can be at the very most tripled. At the time of their estimate approximately 80 billion pounds of fish were being taken. Thus they estimate 240 billion pounds could be harvested. This value is roughly half that of the preceding one. Therefore, the conservative estimate provides FPC supplements for 4 billion people.

It is important to recognize that the estimate of Graham and Edwards relates only to present conditions and present harvesting devices. Aquaculture techniques, new gear, and the like would provide many times the fish harvest they say is now possible.

No matter what production figures we use, there appears to be a significant volume of animal protein available from our world's oceans.

The Bureau of Commercial Fisheries, Technological Laboratory, College Park, Md., has recently completed engineering studies on a solvent-extraction method of FPC production including real cost estimates. Based on a minimum-sized small commercial plant with a capacity of 50 tons of raw material per day, the cost of FPC figures out to be about \$0.18 to 0.20 per pound. This estimate considers fish to cost about \$0.01

a pound and includes a consideration of profit, labor costs, amortization of equipment, etc The only item not considered is by-product oil recovery, which may be a plus factor lowering the final cost of the product The cost of a 10 gram portion of FPC, therefore, would be about one half of a cent The price per kilogram on the basis of 100% protein would be $0 50 Nonfat dry milk on this same basis costs $1 00

If the price quoted is off by a few pennies (and we cannot be expected to tell industry what to charge), the fact is that FPC is the least expensive source of potentially available animal protein We must not forget, too, that the quality of the protein is outstanding—or at least can be, as we have shown from our work at College Park And even though we have difficulty in getting biologists to agree on an estimate of the fishery resource, there is a goodly quantity of fish available for the production of this inexpensive marine protein concentrate

5

General Outlook for Seed Protein Concentrates

MAX MILNER[1]

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Seed proteins, particularly those of the cereals and legumes, are mainstays of human protein nutrition, providing several times more of this nutrient than animal proteins The rapidly growing world protein requirement has directed major attention to the food use of oilseed proteins since these are available in roughly equal quantity to animal proteins Although oilseed proteins are now used mostly for animal feeding or fertilizer, refinements in oil recovery processes as well as new technologies have overcome several problems in the food use of oilseeds, including removal of antibiological factors, toxic plant pigments, and mold metabolites The increasing variety of protein foods of good nutritive quality and appealing gastronomic character from oilseeds suggests that oilseeds will soon contribute significantly to world protein requirements

The serious problem of lagging world food production, and particularly the protein supply, is now widely recognized Altschul (5) has recently written a penetrating analysis of world protein resources, particularly with reference to the possible role which protein materials traditionally unused as human food may play if they can be made available in acceptable edible forms He is one of the first to make the very important point that limitation in traditional thought and attitude seems to have consigned major resources such as the residues of oilseed processing to fertilizer use or at best to animal feeding as optimum means for their utilization The implications of this point provide an excellent approach to a discussion of seed protein concentrates, which is that their use as food and their future impact on the world protein scarcity will be related increasingly to the extent of scientific and technological study devoted to their processing

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and to improved methods of utilization It is only in this light that the potential of seed protein, particularly for direct use as human food, can be properly assessed

The proteins of seeds, particularly those of cereals and legumes, have, since the neolithic agricultural revolution, been the major sources of human protein nutrition Science and technology notwithstanding, the protein from these sources will continue for a long time to come to be our dietary mainstays It is constructive to examine in greater detail the contributions which various traditional resources are making to the world protein balance sheet

World Protein Resources

The cereals contribute in round numbers 75 million metric tons of protein, of which wheat provides 25 million, rice 12 million and corn 20 million tons The legumes, consisting of various beans, peas and lentils, in aggregate, provide, surprisingly, only about 8 million tons Additional but minor plant sources of protein are tubers, including potatoes, and nuts The world animal protein supply, including principally meat, milk, eggs, and fish, has been estimated to be about 20 million metric tons And finally the potential contribution of the oilseeds, which include principally soybeans, cottonseed, and peanuts, can be considered to be about equal to that of all the animal protein now available, 20 million tons per annum Abbott (*1*) has tabulated the contribution to human diets of various protein resources

No single traditional or novel resource, whether increased through agricultural productivity or by new technology, will itself solve the protein problem It is the purpose of this symposium to analyze as carefully as we can the role which each and every resource might play and how this contribution may logically develop

The oilseeds have specific relevance to my topic since at least one of them, the soybean contains, even prior to processing, as much as 40% protein and thus is one of the few plant materials which approach animal products in protein concentration By definition, oilseeds are processed primarily for their edible oil constituent, and this traditional treatment alone, whether by pressing, by solvent extraction or by combination of these techniques results in a residue with relatively large quantities of protein—i e from 30 to 60% (*4*) These, then, are the major types of seed protein concentrates which I will deal with

The quantitative contribution which oilseed protein concentrates could make to the human dietary is about equal to that of all the protein of animal origin being produced in the world today It is pertinent to ask where are these materials being produced in the world and in what quantities? How are these proteins being disposed of now (I would have preferred to have used the term "utilized")? Why have they not been

widely used for human food, and what are some of the reasons for hoping that they will be?

Dealing with the first question, one can point to the fact that soybeans are the world's largest oilseed crop, totaling as much as 35 million metric tons annually, a quantity which is only slightly less than the total production of the next two major oilseeds taken together—peanuts (15 million metric tons) and cottonseed (21 million metric tons) If one includes in the total other significant oil-bearing seeds, such as sesame, sunflower, rape, flaxseed, copra, and palm kernel, the world's production of oilseeds approximates 80 million metric tons (Table I)

Table I Major Oilseeds Used as Food

Species	*Botanical Name*	*Protein Content (Av), %*	*Oil Content (Av), %*	*World Production, 1000 Metric Tons*	*Major Producing Countries*	*Problems in Processing as Food*
Soybean	*Glycine max*	42	20	35 000	U S China	Antitrypsin factors Hemagglutinin factor
Peanut	*Arachis hypogea*	27	48	14,800[b]	India Senegal, Nigeria	Aflatoxin
Cotton seed	*Gossypium*	30	30	20 600[b]	U S, India, USSR, Mexico, UAR, Brazil, Pakistan	Gossypol, malvalic acid, aflatoxin
Sesame	*Sesamum indicum*	25	50	1 500[b]	China, India, Sudan, Mexico	Fiber, oxalate
Sunflower	*Helianthus annus*	30	40	6 840[b]	USSR, Argentina, Uruguay, S Africa, Turkey	Fiber
Coconut	*Cocus nucifera*	8[c]	65	3 200[b]	Philippines, Indonesia Ceylon India, Malaysia	Fiber

Estimated for 1965
[b] (6)
[c] Dried meats (copra)

Soybeans alone account for about 40% of the world's total oilseed crop In 1965, 65% of the world's soybean crop was produced in a single country—the United States The doubling in the world's soybean resources between the immediate post-World War II period and recently is due primarily to the U S crop, which has risen from 300 million to 860 million bushels (23 million metric tons) China is the second major producer (about 8 million tons) and thus the United States and China together produce 90% of the world's crop Other countries producing soybeans in amounts worth mentioning are Japan, Brazil, and Indonesia Unfortunately, this valuable protein resource is virtually unknown in many countries where the need for protein is the greatest

Cottonseed, the next in prevalence, is much more widely distributed than soybeans Most tropical and subtropical areas of the world, which include many of the developing countries, produce cotton, and the production of this seed has shown a moderate increase in recent years Major non U S producers are India, the USSR, Mexico, UAR, Brazil, and Pakistan

Peanut production, like that of cottonseed, is also more spread about than soybeans India and several countries of West Africa being the major producers India alone grows about one third of the world's total crop Sesame, one of the few seed sources rich in the sulfur containing amino acid methionine, is the only oilseed which has decreased in production since the postwar period

As for the current channels of utilization of oilseed protein, precise breakdowns in ratios of use for animal feeding and fertilizer are somewhat difficult to come by, but one general rule exists—very little is either processed or used at present for human food For all practical purposes, about 95% of the U S soybean crop not exported is crushed for oil and the meal is fed to livestock, principally poultry Possibly as much as 2% of the crop is processed for food The percentage of soybean meal produced in the United States which has been exported has risen in ten years from about 2% of the crop to about 12% at the present time Export of unprocessed soybeans has grown by a similar amount Soybeans and their products, including oil constitute the largest single U S agricultural export today Most of these materials go to Western Europe and Japan where a rising standard of living is calling for increased amounts of animal protein in the diet One of the primary reasons for the decrease in availability of skim milk powder for relief feeding from U S donations during the past few years, as well as for concessional sale to individual countries, has been the increased demand in Europe for milk products generally and particularly for producing milk fed veal The high standard of living in the United States and that developing in other industrialized countries (including Japan) is reflected in an almost insatiable demand for animal protein,

whose production is based to a considerable extent on feeding rations supplemented with oilseed protein concentrates For this reason, incidentally, soybean oil has been a surplus commodity under the U S Food for Peace operations, whereas soybeans and soybean meal for which a strong and increasing dollar demand exists, are as yet unavailable in this program

On the U S scene soybean alone accounts for over 55% of the protein concentrates fed to livestock Cottonseed accounts for about 15%, meat by-products 11%, corn gluten about 7%, and fish meal about 4%

We may take India as a typical developing country in terms of the pattern of utilization of oilseed protein by-products There is virtually no soybean production in India Peanut, the largest oilseed crop, about 5 million tons annually, is the mainstay of the country's edible oil supply Peanuts are crushed for oil by obsolescent and inefficient technological means About 10% of the defatted cake may be exported Only insignificant quantities are used as food and little more for animal feeding Informed estimates suggest that 80% of the Indian peanut press cake, containing up to about 40% protein (not to mention residual oil of 10% or more), is used primarily as manure There are obviously both tragedy and challenge here when one considers that this largely vegetarian society, many segments of which suffer from serious protein malnutrition, has developed no effective or rational use for this protein resource

The utilization of cottonseed, the second oilseed crop of India, is also pertinent Because of several factors, including the generally obsolescent oilseed processing technology, a ban on the use of petroleum solvents for food processing, and a conviction among farmers that whole seeds are superior to press cake for cattle feeding, only about 20% of this vegetable oil resource is recovered for human food use

The question becomes urgent Why do these patterns of utilization exist? Why, except for soybeans to a limited extent, have oilseed proteins been used hardly at all as human food? Why indeed, in its painfully pragmatic approach to food selection, even in times of scarcity and starvation, has the human race rejected these traditional protein concentrates as food?

Much of the answer is obvious from a casual visit even to a modern oilseed plant which is processing peanut or cottonseed for oil The procedures employed emphasize the recovery of oil as the primary product while the meal or press cake generally receives scant consideration in terms of safeguarding nutritive and sanitary quality Generally these by-products are fibrous in nature, contain large quantities of hulls and other debris, and in most cases have been overheated to the extent that the protein nutritive quality is severely impaired The point I am trying to make is simple—that every advantage and economy which have been

applied to oil recovery have tended to downgrade those qualities of the protein necessary to provide a palatable, nutritious, and sanitary food

Some recent scientific discoveries have suggested additional reasons why these materials have not been used traditionally as food I refer to the problems of fungal toxicity and intrinsic antibiological factors In retrospect we must acknowledge and indeed endorse the pragmatic wisdom of our ancestors in rejecting materials of this kind as food By the same token, as scientists and technologists we have taken on a rather major challenge in attempting to reverse these age-old traditions and practices

Soybeans

The soybean, containing up to 40% of protein, can be considered as a protein concentrate even without defatting The traditional oriental processing, which involves aqueous extraction of ground beans, tends to retain most of the fat in the final protein curd product The protein nutritive value of the soybean is among the highest of all proteins of vegetable origin (*3*) In comparison with other plant proteins it is unusually high in lysine and is therefore useful as a supplement to the cereals Its limitation in terms of protein nutritive quality may be primarily a moderate deficiency in methionine Breakfast bacon and eggs attest strongly to the nutritive virtue in animal rations of a mixture of corn and soybean meals

The pragmatic methods developed centuries ago by the people of Southeast Asia to process soybeans into a great variety of foods are widely known The major problem, and one which must not be minimized, is the bitter flavor principle of mature beans, which is not easy to remove, particularly by home-processing methods More recently, there has occurred scientific clarification of the variety of deleterious antibiological factors in soybeans apparently primarily protein in nature, most of which are fortunately destroyed by carefully controlled heat processing

Among the major oriental foods of value, whose processing represents a uniquely pragmatic answer to these problems, one can list the tofu (soybean curd) of China and Japan and the variety of fermented products derived from it, particularly in mixtures with cereals, including miso and natto The Indonesian tempeh made by fungal fermentation of soaked soybeans with the mold *Rhizopus*, followed by deep fat frying, has received considerable study in the United States recently All these products are interesting and nutritious foods, and in a few instances such as tempeh, are actually appealing to the western palate Their processing and use as protein foods in a number of countries outside of the Orient merit thorough investigation

Soybean milk, produced by aqueous extraction as the first stage of traditional tofu production, can be a product of considerable merit in its

own right It has never achieved the popularity it deserves even in those cultures where other soybean foods are traditionally accepted, and this I would attribute primarily to the fact that home and village processing are unable to control the critical heat-processing step adequately, and also to the absence of refrigeration which is required to maintain this product safe, as is necessary for fresh dairy products The greatest promise for its expanded use must be predicated on processing it by sterilization on an industrial scale Some urban enterprises of this type are already in operation, notably a flourishing one in Hong Kong In Indonesia a spray-dried soybean extract of this type has been on the market for several years Additional research and development in these processes are necessary in order to improve the economic aspects, the flavor, and the nutritive quality of the product Nevertheless it appears certain that soybean milk in liquid or in powdered forms suitable for reconstitution has a bright future, particularly in the rapidly expanding urbanized areas of Southeast Asia

That the controlled application of steam and heat following solvent defatting of soybean products effects marked improvement in the flavor and feeding value of soybean oil meal, as well as virtual elimination of the deleterious factors, came to be recognized only after the introduction of solvent processing in the United States some 25 years ago Taking into account minimum sanitary standards and food-grade processing requirements, defatted edible soybean meal containing 50% or more of protein has been shown to be an excellent source of economical protein in a form suitable as human food The pre-eminent virtue of these nutritious soybean grits and flours is their low cost, representing probably the greatest value in protein available on today's market If the present U S soybean crop were to be processed entirely into such toasted soybean protein products, instead of being used for animal feeding, where 80 to 90% of protein and calorie substance is lost, it could provide 750 million children with a 40-gram ration of high quality protein per day This calculation may be more than an idle speculation in terms of proposals being discussed in Washington these days to expand U S production of soybean protein and other protective foods to meet the most critical aspects of the world's food shortage

As useful and cheap as defatted, heat-processed soybean meals and flours are as protein supplements, they suffer certain limitations which additional research and process development must seek to remedy In the first place their appearance and flavor remain a compromise among various processing factors related to the solvent-defatting step The subsequent desolventizing of the meal with moist heat causes color development (Maillard reaction), yet heat processing is necessary to inactivate the deleterious antibiological factors Since the protein becomes largely heat-denatured and therefore insoluble, it has serious technological limitations in terms of control or modification of physical properties desirable for

a variety of foods Possibilities for combinations with other foods are also restricted Much challenge to research exists in finding more effective and economical ways to process soybeans as food and particularly to improve their palatability and flavor A negative economic factor in a developing country is that efficient production of a solvent processed product requires considerable capital investment and the installation of expensive ancillary facilities such as grain storage and drying If the United States should decide to make this protein concentrate available in quantity in an expanded Food for Peace program, the problem of a market outlet for oil might again become a serious factor

It is for such reasons that much favorable notice has been given to producing edible full fat soybean flour by a rather straightforward cooking-extrusion technology This approach to edible soybean processing, particularly in terms of providing low cost foods in developing countries, seems to represent a major technological advance

The efforts of the U S soybean processing industry to expand its markets have led to a number of interesting modern products and processes which may in the future achieve considerable economic significance Soybean flour doughs can be extruded and fixed into meatlike forms Soybean protein concentrates containing 70% protein are prepared by leaching of soluble carbohydrate and mineral components of soybean meals by water, dilute acids or alcohol These products because of their blandness and light color are finding their way into many new processed cereal and meat products A considerable demand has been built up over a number of years in the paper industry for industrial grade soybean protein isolates prepared by alkali dispersion of defatted raw soybean flakes Soybean glues are increasingly important Edible forms of the soluble isolates (spray-dried alkali dispersions) are used in a number of sophisticated food applications such as for foaming and gelling agents, and also as the protein component of coffee whiteners, frozen desserts, and soybean beverages The increasing demand in the food industry for modified whey, casein and other high grade dairy by products has stimulated increased interest in these soybean protein products All are of a specialized nature and relatively costly possessing valuable specific properties tailored to a variety of technological needs in U S food processing Their sophistication may appear at this moment to have little relevance to the needs for protein in developing countries particularly in terms of the economics involved On the other hand this basic technology may well find early application in some of these countries—for example to augment the cow s milk supply Furthermore protein isolates are pointing the way to entirely new and highly sophisticated foods which may open mass markets for this product I refer particularly to the production of highly textured meat like materials, prepared by filament-spinning techniques

Cottonseed

Cottonseed suffers from several drawbacks in spite of the fact that its protein biological quality, limited to some extent by methionine and lysine deficiency, is not markedly inferior to that of soybean The most serious problem is the ubiquitous and toxic natural polyphenolic pigment of this species—gossypol—whose presence has until recently restricted even the animal feeding use of cottonseed meals almost entirely to ruminants The other drawback to wider edible use of cottonseed protein concentrates is that it has been a prisoner of traditional processes, which have been concerned only with oil recovery Studies during the past 15 years have greatly clarified the effects of heat processing in terms of the basic mechanism of binding the gossypol to proteins, particularly to the detriment of the lysine content, which is already limited Wider understanding of this problem and adjustment of the traditional operations accordingly can result in marked improvement of cottonseed protein concentrates, many of which are now acceptable for swine and poultry feeding (*2*) As a matter of fact, some of these heat-processed gossypol-bound materials are already in use in Guatemala, Colombia, and Peru for the production of protein-rich cereal food mixtures highly useful as infant weaning products A food-grade product of this type has been marketed in the United States for a number of years Guidelines for producing such edible products by modification of standard oil meal processing have been made available by the United Nations agencies

Fortunately, biochemical and processing studies on cottonseed are continuing at an accelerated pace, aimed at greatly improved methods for producing low-gossypol cottonseed protein concentrates, of considerably higher nutritive quality and palatability than the traditional meals (*2*) A commercial firm in Sicily is simultaneously defatting and degossypolizing cottonseed with acetone Apparently the problem of oil recovery from the acetone miscella has been adequately solved in this process

The Southern Regional Laboratory has developed a mixed solvent technique, based on acetone, hexane, and water, for simultaneously defatting and degossypolizing, which permits removal of the pigment without prior heating of the raw cottonseed flakes The protein quality of this product is essentially unharmed, the lysine being virtually fully available The nutritive quality of the protein remains very close to that of a properly processed soybean flour This product has already been tested by feeding to young infants as a sole source of dietary protein, with excellent results In further exploration of this technology, the USDA group has shown that aqueous acetone by itself can selectively remove gossypol, and the way now seems open to low-gossypol meals in which much of the nutritious cottonseed oil is retained Engineering processes of this type are also under study in Hyderabad, India

There seems little doubt that techniques of this kind have been perfected to the point where they can be applied quickly on a commercial scale if and when the demand for such products appears Cottonseed production is widely distributed in a number of developing countries which suffer from protein scarcity It is my prediction that these new processes will provide the protein feeding component necessary for expanded production of swine and poultry—i e , an animal protein economy, in these regions Incidentally, the solvent degossypolizing techniques can provide essentially raw protein and thus this method opens the way for producing protein isolates as a logical development, following the soybean protein model

Another remarkable advance in cottonseed protein quality has resulted from a recent breakthrough on the genetic front Gossypol-free varieties of cottonseed have been developed, from which the characteristic pigment glands are virtually absent (*2*) This breeding factor can apparently be introduced into virtually any strain of seed without impairing the fiber quality or desirable agronomic characteristics The cottonseed meals and flours are considerably lighter in color and of excellent nutritive value The National Cottonseed Processors Association has developed an active program of seed production and distribution, which should make these new varieties widely available to growers by 1970

Peanut

The third major oilseed resource peanut, which suffers least from indigenous or naturally occurring deleterious components, has nevertheless recently weathered a rather difficult and unsettling experience in terms of the problem of aflatoxin (*8*) The recent identification of this fungal-induced toxicity which can occur on this and other crops including cottonseed when improperly harvested and stored, may indicate another major reason why as a vegetable oil industry by-product, peanut meal, has achieved little traditional food use

In any event, with means to control the aflatoxin problem at hand, this major protein resource will doubtless achieve wider use for feeding as well as for industrial purposes (7) Careful selection of peanuts can provide flours of good nutritious quality, highly valuable in a variety of diets and acceptable as components of low-cost protein-rich foods suitable in a number of developing countries

I have tried to indicate the impact which seed protein concentrates could make on the world protein needs if they were processed and used more extensively in human foods As a result of several new technologies, more valuable and sophisticated ways to use them are now available Nevertheless the art, and to a far greater extent the technology, of their

processing and use as food still lag very far behind our knowledge and art in the use of traditional foodstuffs such as cereal grains, dairy foods, and the animal proteins On the basis of the considerable expansion of the study of nutritional and food technology now under way, it seems certain that these products will soon take their place as major resources in the world's food protein balance sheet

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Discussion

B F Buchanan It is an interesting and significant observation that there is potentially as much protein available from today's oilseed production as from all edible animal products

Equally significant is the observation that no single traditional or new protein resource will itself solve the problem of the world dietary protein insufficiency It is perhaps fortunate that the latter is, indeed, *true in view of the fact that no single vegetable protein* has the proper amino acid composition for optimum nutrition Since carbohydrates and fats provide a much more economical source of calories than does protein, it is highly important that insofar as possible, proteins should be utilized for their contribution to the diet as *proteins* and not squandered

as calories Therefore, to get the most mileage from those vegetable proteins available, consideration should be given to combinations from those various sources which yield optimum amino acid balance, thus avoiding the risk of expending valuable amino acids for calories alone in the protein marginal diets

Now let us consider some of the data presented by Dr Milner Current annual production of oilseed of 80 metric tons represents a potential of about 45 billion pounds of protein At the conservative estimate of feeding only 55 grams of protein per person per day, this 45 billion pounds of protein would feed only about one billion persons for one year With two-thirds of the world's population suffering from protein poor diets, this quantity of protein from today's oilseed crop would satisfy only about one-half of that protein deficient population Although this is a tremendous raw material resource and should not be minimized the fact that there is not enough to satisfy today's demands is again perhaps fortunate since it virtually forces one to mix protein sources to achieve adequate supplies, thus resulting in finished food products of better nutritional quality

If one could satisfy the requirements of an adequate supply of nutritionally balanced proteins to feed the world, two important problems would still have to be overcome First is the matter of developing acceptable food products familiar to the tastes of the population in the country under consideration Dr Milner mentioned the great waste in many areas of currently produced oilseed proteins as fertilizers and as animal feeds This waste is attributable primarily to emphasis on oil recovery at the expense of retaining quality in the protein fraction But let us assume edible-quality production Before man will eat these proteins they must be fabricated into acceptable and palatable food products A hungry person suffering from a protein deficient diet most frequently lacks the tools to prepare a meal from soy flour, cottonseed flour, peanut flour Those tools are not necessarily just the hardware for preparation and fuel for heating, but more often the knowledge to help him build attractive finished foods, ready to be eaten with a minimum of preparation

Scientifically advanced nations today possess the technologies to produce and to convert protein resources into nutritious, palatable, and acceptable food products Ample evidence is exhibited for example, in the fact that the United States uses upwards of 50 million pounds of soy protein per year as ingredients in such items as bakery products, meat products, breakfast cereals baby foods milk like products and a host of special dietary foods

Second, even the most nutritious, acceptable food product has to be sold to the consumer Marketing is a highly developed skill, successfully used in this and many other countries to create awareness in the mind of the consumer

Therefore, to relieve the problem of world protein malnutrition it is necessary (a) to develop food products indigenous to the population—products that are nutritious, stable, easily prepared for eating, and highly acceptable, (b) to utilize to the fullest our marketing talents, even intrigue if necessary, to bring these products to the attention of those most in need, (c) finally, to use every skill and incentive at our command to encourage these peoples to work and pay for what they get

6

Production of Lysine and Methionine

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Lysine and methionine are manufactured for use as food supplements in human nutrition and animal feeding The larger part of present production is feed grade methionine L-Lysine is made by fermentation employing selected strains or mutants of microorganisms growing in a solution of glucose or molasses, ammonium compounds, inorganic salts, and other substances DL-Methionine is synthesized from acrolein, methyl mercaptan, and any readily available sources of cyanide and ammonium ions Unlike lysine, both the D and the L-forms of methionine can be utilized by the body hence resolution is not necessary A very large demand for lysine, methionine, and other amino acids may be created by the proposed supplementation of the diet in protein-deficient regions The necessary production is within the capability of the chemical industry

Each of the major industrial countries of the world engages to some extent in the production of essential amino acids At present only lysine and methionine are manufactured in commercial quantities and, of the two, methionine is produced in the larger amount The production of lysine in large volume has lagged for the lack of a sufficiently large market, and the demand in turn, has been slow in developing because of the relatively high price The widespread world food shortage, and the protein deficit in particular, may create an urgent demand for lysine, methionine, and certain other essential amino acids to supplement and upgrade the incomplete proteins of the cereals pulses, and oilseeds that constitute a large part of the diet in the developing countries of Tropical America, Africa, and Asia Anticipating this demand Japan has been particularly active in research on the production of amino acids by fermentation

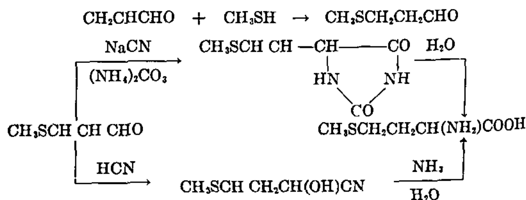
Lysine and methionine as well as most of the other amino acids can be made either by chemical synthesis or by the culture of microorganisms on suitable substrates Methionine is currently produced by synthesis while the fermentation method for lysine has supplanted the chemical syntheses

formerly used Production by microorganisms is the more economical method for lysine because it yields only the levo enantiomorph, the form in which lysine occurs in nature and is required for nutritional purposes With methionine, however, both the dextro- and the levo- forms can be utilized by the body, hence resolution of the racemate obtained by chemical synthesis is not necessary

Methods of Synthesis

Various synthetic routes are available for producing amino acids, but he route most commonly followed in manufacturing lysine and methionine by chemical synthesis involves the Bucherer modification of the Strecker synthesis In this route an aldehyde, on reaction with $(NH_4)^+$ and $(CN)^-$, yields a hydantoin which, on hydrolysis, yields the amino acid The ultimate raw materials required are petroleum and/or coal, inorganic salts, sulfur, and atmospheric nitrogen All of the raw materials and most of the intermediates, as well, are in abundant supply The quantities of raw materials that would be required for the largest production that can be foreseen would amount to only a small fraction of the quantities now utilized for other purposes

Methionine The present commercial synthesis of methionine starts with acrolein (*25*) Successive steps are reaction of acrolein with methyl mercaptan to yield 3-(methylthio)-propionaldehyde, reaction of this aldehyde with sodium cyanide and ammonium carbonate, or other sources of cyanide and ammonium ions, to yield 5-[(2-methylthio(ethyl)] hydantoin, and hydrolysis of this hydantoin to yield methionine Alternatively, the 3-(methylthio)-propionaldehyde may be made to react with hydrogen cyanide to yield the corresponding 2 hydroxynitrile, the hydroxyl is then replaced by an amino group by reaction with ammonia under pressure, hydrolysis of the nitrile then yields methionine The reactions by the two routes are shown diagrammatically as follows

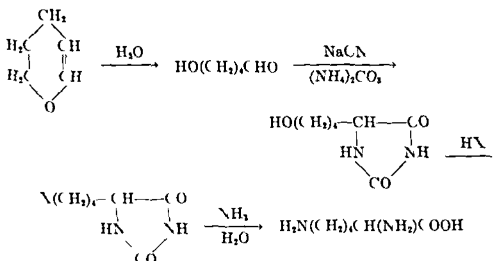


The acrolein required in this synthesis can be made by oxidizing propylene obtained in petroleum refining Methyl mercaptan may be

obtained by reaction of methanol and hydrogen sulfide, and hydrog cyanide, by reaction of ammonia and methane

If the hydroxyl of the 2 hydroxy-4-(methylthio) butyronitrile is n replaced by an amino group the nitrile yields, on hydrolysis, the hydrox analog of methionine This product, in the form of its calcium salt, manufactured in quantity for use in animal feeding where it serves th same nutritional functions as methionine

Lysine Lysine can be made synthetically from a variety of inte mediates, including dihydropyran (*18, 20*), caprolactam (*2, 21*), piperidi (*22*), and cyclohexanone (*4*) In the synthesis of lysine from dihydropyra the first step is opening of the ring by hydration to yield 5 hydroxyvalera dehyde This aldehyde, on reaction with sodium cyanide and ammoniu carbonate or other sources of cyanide and ammonium ions, yields 5 (4-hy droxybutyl) hydantoin The hydroxyl group of this hydantoin is replace by a halogen on reaction with hydrogen chloride or hydrogen bromid The halogen, in turn, is replaced by an amino group on treatment wit ammonia, and the hydantoin on hydrolysis yields lysine Following is sketch of the reactions



Resolution of the DL-lysine obtained by chemical synthesis may b effected by reaction with an optically active acid selected so as to form tw diastereoisomers that differ sufficiently in solubility to permit separation b fractional crystallization Among the acids which have been proposed fo this purpose are L-glutamic (*3 17*) D-camphoric (*26*) and, D homocysteini acid (*7*) With the first of these the L-lysine L-glutamate separates ou from a water alcohol mixture and on treatment with hydrochloric acid th lysine is obtained as the monohydrochloride and the glutamic acid i recovered The unwanted D lysine is recovered as the monohydrochlorid from the filtrate from the L-lysine L-glutamate and is converted b heating with alkali to the racemate which is then recycled in the process

Biochemical Production

The chemical synthesis of lysine has been supplanted by fermentation processes in which selected microorganisms are grown in a broth containing a nutrient such as glucose or molasses, together with an ammonium compound, small amounts of inorganic salts, and other materials necessary for the growth of the microorganisms, notably biotin and small amounts of certain amino acids. Lysine accumulates in the broth, together with other amino acids in smaller amounts, vitamins and other substances. The lysine may be recovered conveniently by passing the filtrate from the broth through a column of an acidic ion-exchange resin which takes up the lysine. The lysine is eluted from the resin by a solution of ammonium hydroxide. On evaporation of this solution to dryness the ammonia volatilizes, leaving a residue of lysine which is converted to the monohydrochloride, and this, in turn, is purified by recrystallization.

The efficiency of the fermentation process depends on selecting a strain of microorganisms capable of producing a relatively high concentration of lysine in the broth. Not only have thousands of naturally occurring organisms been investigated, but in addition mutant strains of various organisms have been produced by irradiation with cobalt-60 and with x-rays.

The economic advantage of the fermentation process lies not only in the direct production of the desired L-isomer of lysine, but also in the use of cheap by-products such as molasses and corn-steep liquor as the nutrient.

A typical lysine fermentation is described by Kinoshita in a review on the production of amino acids (*12*). The organism employed was a special strain of *Micrococcus glutamicus*.

A graph relating the production of lysine to the disappearance of glucose showed the maximum accumulation to be about 2.5% of lysine, expressed as the monohydrochloride.

The industrial production of L-lysine in Japan, the United States, and France, according to Kinoshita (*12*), employs special strains of *M. glutamicus* growing on a culture medium containing molasses as the principal nutrient. Molasses is preferable to pure glucose because it contains biotin, other vitamins, and small amounts of certain amino acids, all of which are necessary or desirable for the growth of the microorganisms. While Kinoshita states that it is not unusual to obtain a broth containing 2% of lysine, lower percentages are more commonly reported in experiments described in the literature. The yields in commercial production apparently have not been disclosed.

The development of fermentation processes has been greatly aided by research on the ways in which amino acids are formed in nature. Present knowledge of the biosynthesis of lysine is summarized as follows in a recent book by Meister (*15*).

Two distinct pathways are known that lead to the synthesis of lysine In certain fungi and algae the carbon skeleton of lysine arises from acetate and alpha ketoglutarate by a biosynthetic sequence that includes alpha aminoadipic acid The other pathway has been found in bacteria, higher plants blue-green and green algae and certain fungi, in these organisms, the lysine carbon chain is synthesized from pyruvate and aspartate, and alpha, epsilon-diaminopimelic acid is the key intermediate At the time of this writing more is known about the diaminopimelic acid pathway

Some fermentation processes are conducted in two stages, the first leading to the production of aminoadipic acid or diaminopimelic acid, and the second being the enzymatic conversion of these acids to lysine In one such process (*14*) a mutant of *Escherichia coli* was used which, itself, required a low concentration of lysine for its growth When an inoculum of this organism was added to a culture medium containing 4% of sugar beet molasses and 3% of glycerol as nutrients, diaminopimelic acid accumulated to the extent of 0 7% Some back-mutation of the organism evidently occurred since on addition of toluene to rupture cell walls, sufficient diaminopimelic acid carboxylase was released to convert the diaminopimelic acid completely to lysine on standing

World Production of Amino Acids

Statistics regarding the production of amino acids seem to be limited to those collected and reported by the U S Tariff Commission in connection with the production and sale of synthetic organic chemicals in the United States Lysine, methionine, and other amino acids except glutamic are classified as medicinal chemicals, while glutamic acid, as monosodium glutamate is shown as a flavor and perfume material because of its use as a condiment

Data on the production sales, and value of amino acids since 1945 are summarized in Table I The maximum annual production shown is 5 946 000 pounds, or a little under 3000 tons for 1961 In 1961 the detailed statistics show the sales of lysine as 296,000 pounds, or slightly less than 150 tons Since 1961 too few firms have been making lysine to admit the separate reporting of production or sales figures, inasmuch as so doing might disclose the output of individual firms For the same reason the hydroxy analog of methionine prior to 1964 was not shown as a separate item but was blanketed in a general ' all other ' item In a preliminary report for 1964 however, methionine and the hydroxy analog are grouped together with a total production of 6,049,000 pounds When this figure is compared with the total production of amino acids for 1963 in the amount of 3,056 000 pounds it is evident that the hydroxy analog is made in approximately the same amount as methionine itself

Inasmuch as lysine and methionine are the only amino acids manufactured in quantity, it is possible to make a rough estimate of the amounts

Table I Production and Sales of Amino Acids in the United States (*24*)

Year	*Production, 1000 Lb*	*Sales, 1000 Lb*	*Value, $1000*	*Value, $ per Lb*
1945	177	189	502	2 66
1950	929	736	1 942	2 65
1955	1 773	2,007	4,901	2 44
1960	5,163	4,214	6,330	1 50
1961	5,946	5,106	7 320	1 43
1962	5,007	5,492	7 210	1 31
1963	3,057	3,283	4,256	1 30
1964	6 049[a]	5,860[a]	6 203[a]	1 06[a]

[a] Preliminary report on methionine and hydroxy analog (*24*) In previous years the hydroxy analog was included in an all other' item In 1964 amino acids other than methionine and glutamic acid are apparently included in an "all other' item Glutamic acid and monosodium glutamate are shown as an item in the class of flavor and perfume materials

of each that are sold in the United States on the basis of market quotations and the total value shown in the Tariff Commission report In 1963 the price of feed-grade methionine was $1 10 to $1 17 per pound, depending on quantity, and the price of lysine was $3 90 per pound Thus the sales at an average price of $1 30 per pound show that probably more than 90% of the production for sale in the United States was feed-grade methionine

Figures are not available for amino acids production in European countries, Canada, and Japan Kinoshita (*13*) estimates that 3000 to 5000 tons of lysine per year can be produced in Japan, though this rate has not yet been achieved The Netherlands does not yet produce amino acids in quantity, but plans have been made to manufacture lysine from capro lactam at the rate of about 3000 tons per year, beginning in 1967 (*19*)

Statistics on imports into the United States show amino acids as a separate category The amounts of the individual amino acids are not shown, but quantities and values such as those given in Table II indicate in some cases which amino acids constitute the bulk of the imports from each country At $47 50 per pound the imports from the United Kingdom could only be amino acids other than lysine and methionine The small item from Switzerland in the first half of 1965—22 pounds valued at $2932—points to this country also as a source of amino acids that are not yet manufactured in quantity Imports from other countries at prices of less than a dollar a pound could be only feed-grade methionine or possibly its hydroxy analog No confusion could arise from glutamic acid since monosodium glutamate is reported in a separate category

Possible Future Demand for Amino Acids

The major prospective use of amino acids is for supplementing the incomplete vegetable proteins in the diets of the peoples of the food

deficient regions of America, Africa, and Asia (*1, 5, 6, 8–11*) The limiting amino acids to be supplied would be not only lysine and methionine but also threonine, tryptophan, and possibly still others The quantities that would be required for effectively upgrading the diet in any one of the protein-deficient regions would be a great many times the present total production

The widespread interest in supplementation is shown by the large number of papers in American and foreign scientific periodicals on the use of amino acids both for animal feeding and in human nutrition The number of patents that are being issued in the major industrial countries on the production of the different essential amino acids gives evidence of commercial interest in the subject In the United States the scale and extent of work in progress are shown by the significant imports of the amino acids that are not yet in commercial production

Table II United States Imports of Amino Acids for Consumption (*23*)

Country	*1964*			*January June 1965*		
	Quantity Lb	*Value $*	*Value $/Lb*	*Quantity Lb*	*Value, $*	*Value $/Lb*
Canada				5 000	3 494	0 70
Denmark	1 102	2 860	2 60			
United Kingdom	3 824	181 693	47 50	6	1 361	227
Netherlands	111 500	77 984	0 70	97 026	64 978	0 67
France	122 101	112 650	0 92	23 963	22 742	0 95
West Germany	154 477	150 225	0 97	47 259	44 717	0 95
Switzerland	6 458	8 008	1 24	22	2 932	133
Israel		3 695			3 401	
Italy	6 171	11 439	1 86	5 006	10 339	2 05
Japan	117 001	323 364	2 76	76 600	163 595	2 13
Total	522 634	871 918	1 67	254 882	317 559	1 25

The subject of supplementation is no longer of only academic interest A proposal is under consideration in government circles to add lysine to the wheat that is being sent to developing countries under Public Law 480 This addition would have a twofold advantage The lysine would double the efficiency of the 10 to 14% of protein in the wheat and would thereby compensate for a significant part of the deficit in animal protein in the diet of the recipient countries—a deficit that cannot in the foreseeable future, be supplied as milk, meat or eggs Furthermore, the central control of the wheat would make the addition of lysine a relatively simple matter, assuming that means can be developed for incorporating lysine in wheat in a way that would insure its being carried over without loss into bread and other products made from the wheat

Incidentally, bread enriched by adding 0 25% of lysine is now made and sold in the United States by a large chain of food stores, notwithstand-

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The major prospective use of amino acids is for supplementing the incomplete vegetable proteins in the diets of the peoples of the food

ing the fact that, on the average, there is an abundance of adequate protein from other sources in the American diet

The shipments of wheat under Public Law 480 in 1964 amounted to 563 million bushels or 33,780 million pounds (*16*) The addition of 0 25% of lysine, for example, to this quantity of wheat would require 85 million pounds or 38,800 metric tons of lysine, a quantity that is at least 10 to 20 times the present world production The cost of lysine at the present market price of $3 90 per pound would be $0 58 for each bushel of wheat treated, or about one third of the cost of the wheat This cost would almost certainly be regarded as prohibitive by administrative officials for other than a pilot operation If, however, the cost of lysine could be reduced to $1 00 a pound with large scale manufacture, the lysine for treatment of the wheat would cost only $0 15 per bushel, or about 2 5 cents for each pound of protein that would be upgraded The estimate of $1 00 a pound is based on the usual economies that attend large scale manufacture, with computer-controlled continuous processes The cost of lysine might be further reduced by using crude lysine from the fermentation, without separation from the vitamins and the other amino acids produced by the fermentation since these substances might prove useful additions to the diet as well as the lysine itself

The initiation, by national or international action, of a program involving the large scale supplementation of staple foods would present a twofold challenge to the chemical industry The challenge would be, first, to produce lysine, methionine, threonine, tryptophan, and perhaps other amino acids in an aggregate quantity of one or two million tons a year at prices that would encourage rather than minimize their use, and, second, to develop flavors, odors, colors, and other characteristics in the products serving as carriers of the amino acids that would make supplemented foods eagerly sought after by the recipient peoples

The chemical industry of the world is fully capable of meeting both challenges Production of the quantities of the amino acids that would be called for would not tax an industry that produces many items in amounts of over a million tons a year In the United States alone the production of synthetic organic chemicals amounts to about 34 million metric tons a year Furthermore, the field of organoleptics has developed to the stage at which the identification and synthesis of substances responsible for the taste and odor of food, if not already accomplished, can be accomplished without major difficulty

The time is ripe for developing a greatly expanded amino acid industry The basic raw materials are available A vast amount of fundamental research is recorded in the literature In every major industrial country there are manufacturers who have had some experience in making one or more amino acids, and doubtless many firms have new processes awaiting an opportune time for development In the course of normal commercial

operations this development would proceed at a deliberate pace The production, for example, of the 85 million pounds of lysine a year, which has been suggested for the wheat being shipped to India, might require a lead time of several years and firm commitments for a continuing market The world food situation today, however, is critical and calls for an emergency program such as that which brought the large and successful synthetic rubber industry into being in two years during World War II With today s superior resources amino acid production could be expanded manyfold in a short time if accorded the requisite priority, funds, and manpower

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Cottonseed Protein in Human Foods

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Cottonseed protein has been a recognized human food, used by people of low and high economic resources as supplement to the daily diet, since vegetable protein mixtures were developed at INCAP to eradicate protein malnutrition. Up to 38% has been used to enrich cereal flours, noodles, flaked breakfast foods, sausages, and other foods. The nutritional value, stability, tolerance, acceptability, and shelf life of these foods are good. Information is presented here on the newer and modified older technologies for producing edible cottonseed protein concentrate, and the fate of gossypol after cooking in mixtures containing cottonseed flour in the presence and absence of calcium and ferrous ions and after ingestion by experimental animals and children.

Cottonseed protein became a recognized food for man 6 years ago, when protein rich food mixtures containing cottonseed flour began to be commercially produced, distributed, and consumed. Although the number of people using cottonseed protein as food is relatively small at present the prospects of increasing its use are great. Increased consumption will follow as more knowledge about its industrial preparation is obtained, preserving its nutritive value, keeping its cost low, diversifying its applications in food, and understanding factors which have limited its use.

The developments which led to the use of cottonseed protein concentrate as human food are based on extensive work on the use of cottonseed meal in animal feeding, particularly for monogastric animals. The information obtained from animal feeding studies can be summarized as follows:

The most important factor limiting the use of cottonseed meal was the presence in the meal of the pigment gossypol. This substance, a polyphenol, is toxic to monogastric animals, particularly swine (*40, 51, 77*).

Cottonseed protein is deficient in the amino acid lysine. This deficiency increases as lysine is bound during processing for oil extraction. The pigment gossypol was also partially responsible, since it reacts with the

ε-amino group of lysine, making it unavailable. The oil is still economically more important, but the technology is progressing, and both the oil and the protein are being emphasized. The difference in their economic value is becoming smaller (*1, 23, 53, 56, 61*).

A further drawback was the fact that eggs from hens fed cottonseed meal develop a dark unpleasant color of the yolk and a pink discoloration of the white, and the membrane separating white from yolk becomes more permeable. These effects were due not only to gossypol but also to the presence of sterculic and malvalic acids in the lipid moiety of the seed (*36, 55, 65*).

The urgent need to develop economic, locally available, and highly nutritive foods for the extremely large number of people, particularly children, suffering protein-calorie malnutrition, moved INCAP to test the possibility of using cottonseed protein for this purpose. Research carried out during the past 8 years at INCAP has resulted in developing several vegetable protein foods containing from 9 to 38% cottonseed protein concentrate, making it possible to use this protein source as a food for many undernourished people of the world.

Table I shows the commercial yearly production of two vegetable protein mixtures (INCAP No. 9 and No. 15), known as Incaparina since 1961. Although their annual production remained essentially the same during the first two years, it doubled in 1963 and increased approximately five-fold in 1964. In 1965 total production reached about 4.0 million pounds. On this basis, in the last 5 years, 800,000 pounds of cottonseed protein have been produced in Central America for Incaparina production and about 700,000 pounds in Colombia.

Table I. Production of Incaparina in Latin America
(Pounds)

	Trimester				
Year	*First*	*Second*	*Third*	*Fourth*	*Total*
1961	—	44 250	100,755	100,731	245,736
1962	68 772	9 568	128,515	—	206,805
1963	63,134	154,464	106 977	163 995	488,570
1964	475 055	237 411	722 844	728 034	2163,344
1965	720 458	832 648			

Not produced because of low availability of cottonseed flour.

) *Availability of Cottonseed and Cottonseed Protein*

Cotton and man have been wedded amicably and profitably since ancient times. The truth of that statement becomes evident on observation of Table II, where production figures in specific countries and the world are shown (*76*). Total world production for the year 1962 was estimated at 23,390,000 tons, showing continuous increases throughout the years. It was

grown at first for its fiber, and later cotton became an important source of cottonseed oil. Because of its world wide cultivation in tropical and subtropical areas and increasing production, cottonseed is likely to become a major and important source of protein for the ever increasing world population. Furthermore, as shown in Figure 1, cotton is grown in most areas where protein-calorie malnutrition is prevalent, offering an excellent opportunity to use its protein to fight this disease.

Although about 75% of the world's supply of cottonseed is processed to obtain oil and meal, in the majority of cases, the meal is not of a grade suitable for human consumption. Until very recently, there was no convincing evidence of its value in human nutrition, although cottonseed meal is one of the commonest protein supplements for dairy and beef cattle and sheep. Limitations which must be considered for effective utilization of this product in rations for swine and poultry either do not apply or are of minor importance for ruminant animals. For feeding monogastric animals cottonseed meal is still used in very limited amounts.

Cottonseed protein in the form of a flour is now available to a limited extent for use as human food, but a protein isolate from cottonseed is not yet commercially available. Very little cottonseed protein is available for human consumption, mainly because until recently cottonseed protein was not recognized as a suitable human food as were soybean, peanut, and sesame. A second limiting factor is that the technology for preparing cottonseed flour for human use is poorly defined, even though cottonseed products of high quality are now available (*24*).

Tentative quality and processing guides for producing high quality edible cottonseed flour for human consumption are given in Table III. The product should have a minimum of 50% crude protein with a level of 3.6 grams of available lysine per 16 grams of nitrogen and a maximum value of 0.060% free gossypol (*63*). It is feasible and desirable to produce material equal to or better than indicated by the specifications.

Cottonseed flour has several advantages for protein enrichment of foods and as an ingredient in protein rich food mixtures. It has a relatively high amount of protein of acceptable quality, and is grown in practically all the areas of the world where there is a deficit of food and protein-calorie malnutrition prevails. Since it is grown for its fiber and oil, the residual protein from its seed is economical in comparison with other oilseeds. By using the technology available today and with the developing technologies cottonseed can yield a product highly adequate for human use.

Nutritive Value of Cottonseed Protein

Amino Acid Composition. The amino acid composition of a protein is the most important factor determining its nutritive quality. The essential amino acid pattern of cottonseed protein in the form of flour is

Table II Cottonseed Production in Specified Countries and the World

Continent and Country	Year Beginning Aug 1[a]		
	Aver 1955–59[b]	1961[b]	1962[b]
	1,000 tons	1,000 tons	1,000 tons
North America			
United States	5,370	5,978	6 096
El Salvador	72	124	144
Guatemala	32	77	125
Honduras	5	9	13
Mexico	975	958	1 140
Nicaragua	94	130	166
Haiti	3	3	3
Total[c]	6 555	7 295	7,700
South America			
Argentina	275	253	303
Brazil	715	1,212	1,104
Colombia	82	187	200
Paraguay	22	23	26
Peru	277	320	342
Venezuela	15	16	19
Total[c]	1,395	2 015	2 000
Europe			
Greece	138	228	204
Italy	24	13	10
Spain	100	233	245
Bulgaria	37	21	24
Total[c]	325	515	500
S S R (Europe and Asia)	3,240	3,410	3 215
Africa			
Egypt	965	823	1 122
Sudan	267	515	454
Angola	18	10	18
Chad and Central African Republic	83	62	103
Congo, Leopoldville	124	40	42
Mozambique	83	95	94
Nigeria	84	84	145
Rhodesia and Nyasaland	6	13	15
Tanganyika	67	71	89
Uganda	157	78	153
Total	1 920	1,880	2 340

Table II Continued

Continent and Country	Years Beginning Aug 1		
	Aver 1955–59[a]	1961[b]	1962[b]
	1,000 tons	1,000 tons	1,000 tons
Asia and Oceania			
Iran	163	283	227
Iraq	24	21	20
Syria	235	305	370
Turkey	376	484	561
China Mainland	4 010	2 800	2 910
Afghanistan	39	45	45
Burma	41	48	48
India	2 235	2 268	2 520
Korea South	29	21	15
Pakistan	697	743	816
Thailand	23	31	29
Australia	2	3	4
Total	7 910	7 140	7 635
World total	21 345	22 255	23 390

Years of harvest
[b] Preliminary
Includes estimates for minor producing countries
Foreign Agricultural Service United States data compiled from official reports data for other countries calculated from lint production estimates
Taken from Ref 76

shown in Table IV (1) It compares favorably with the amino acid requirement pattern for human adults although it is slightly low in lysine and high in leucine Likewise, cottonseed protein contains a relatively large proportion of nonessential amino acids particularly glutamic acid Of the total nitrogen around 30% comes from essential and 70% from the nonessential amino acids The nutritional significance of this is not well studied, animal proteins contain about 50% of their nitrogen as essential amino acids

Protein Value in Experimental Animals Compared with the extensive work reported on the meal, little nutritional information is available on cottonseed flour *per se* or cottonseed protein Moreover, most published research has been done on flour produced before high quality meals were available There is little difference between meal and flour processed by advanced methods to reduce free gossypol and retain protein quality For

this reason, nutritional information already available on meal can usually be applied to predicting the performance of a flour of similar quality



Figure 1 Protein calorie malnutrition and cottonseed production in the world
//// Protein-calorie malnutrition
● Cottonseed

The results of several workers are summarized in Table V Allison *et al* (*2*) reported on the nutritive value of cottonseed flour, with short-term feeding experiments and long-term studies on reproduction and lactation The nitrogen efficiency ratios (*NER*) reported were much lower for the cottonseed flour than for casein when the diet contained 2% nitrogen When the dietary nitrogen was increased to 4%, the difference between the efficiencies for casein and cottonseed flour was reduced The reproduction and lactation of rats fed cottonseed flour at the 4% nitrogen level were normal Although the offspring grew at a slower rate when fed cottonseed flour than when fed casein, the difference became insignificant as the animal matured The nitrogen balance index (NBI) of cottonseed flour in adult dogs was reported by Allison *et al* (*2*) to be 0 60, while supplementation with lysine and methionine increased it to 0 85 Workers using chicks, turkeys, swine, and rats have also indicated that cottonseed flour is limiting in lysine, methionine, and possibly threonine (*8, 30, 41, 60, 61, 64, 69*)

Protein Value in Human Subjects Frenk (*37*) and Cravioto *et al* (*26*) in studies with malnourished children reported equal absorption of

Table III Tentative Chemical Quality of Cottonseed Protein Concentrate for Human Consumption

Moisture, % (maximum)	10 0
Crude fat, % (maximum)	6 0
Protein % (N × 6 25 minimum)	50 0
Crude fiber, % (maximum)	5 0
Total gossypol, % (maximum)	1 2
Free gossypol, % (maximum)	0 06
Free fatty acids (maximum, as % of oil)	1 8
Available lysine, g /16 g nitrogen (minimum)	3 6

nitrogen for the cottonseed protein and milk protein tested A mixture of cottonseed and corn flour fed at 2 to 5 grams of protein per kg was not adequate for the recovery of the malnourished children studied At this intake, nitrogen retention as percentage of intake was around 29% for milk, about 18% for cottonseed flour alone, approximately 17% for a mixture of cottonseed flour and rice, and around 30% for a mixture of equal parts of milk and cottonseed flour

Kaye *et al* (*49*) reported on nitrogen balance data on three babies fed cottonseed flour alone and cottonseed flour mixtures with rice or wheat protein providing half of the protein from last sources Retention with milk was significantly greater than with cottonseed, and the cottonseed rice mixture was superior to the mixture of cottonseed flour and wheat They concluded that cottonseed flour was a reasonably good protein source

Table IV Amino Acid Composition of Edible Grade Cottonseed Protein Concentrate (*1*)

Amino Acid	*G /16 G N*	*Amino Acid*	*G /16 G N*
Lysine	3 30	Aspartic acid	8 01
Leucine	6 46	Glutamic acid	15 99
Isoleucine	2 42	Serine	3 77
Threonine	2 46	Proline	2 68
Valine	3 25	Glycine	2 75
Phenylalanine	4 17	Alanine	4 13
Tryptophan	0 86	Tyrosine	2 53
Methionine	1 40	Histidine	1 98
Cystine	1 45	Arginine	7 15

The results of other workers are summarized in Table VI DeMaeyer and Vanderborght (*27, 28*) reported the percentage absorption, biological value (BV), and net protein utilization (NPU) of cottonseed flour in children to be 87, 62, and 54%, respectively They concluded that cottonseed flour was safe had a relatively good nutritive value and was a possible source of protein in the prevention of protein malnutrition Snyderman

Boyer, and Holt (*72*) evaluated cottonseed protein in premature infants, and showed it to have a net protein utilization of 41% when corrected for nitrogen lost in feces The biological value at protein intakes of 5 and 7 grams per kg of body weight, was 57 and 46%, respectively Cow's milk fed at an intake of 5 grams of protein per kg of body weight had a biological value of 79%

Table V Nutritive Value of Human Grade Cottonseed Protein Concentrate on Several Experimental Animals

	Biological Method			
Diet	*PER* (*rat*) *10%**	*NPU* (*rat*) *10%**	*NBI* (*dog*)	*Growth* (*chick*) *20%**
Cottonseed protein	2.14	50	0.60	201
Cottonseed protein + lysine	2.40	—	0.72	308
Cottonseed protein + lysine + methionine	—	—	0.85	303
Casein	2.38	72	0.78	—

* Protein level

Methods of Processing The industrial methods of removing oil from cottonseed are essentially the same as those for processing other oil containing seeds Because of the demand for better extraction methods, better quality of oil, and better quality meal, important technological modifications have been developed and introduced and newer and more efficient technologies have evolved The details have been described (*3, 33*) and only conditions directly affecting nutritive value are mentioned in this report The first industrial method for processing cottonseed, hydraulic press processing, is still used in certain underdeveloped areas of the world Although screw press processing is the most common method, treatment of the seed by these two processes is essentially the same The screw press processing method is more efficient for oil extraction than the hydraulic press method, but the latter is less damaging to the protein quality of the seed

Much work on the utilization of cottonseed protein in the form of meal and on its nutritive value was performed using the by product of cottonseed obtained by these processes It was soon found that the nutritional value the protein of such products was of very low quality, because of excessive ...rature, pressure, and time used in processing Examples of the ... of commercial processing on the nutritive value of the protein of cottonseed meal have been reported by Milligan and Bird (*59*) using chicks and by Horn *et al* (*42*) using rats These investigators reported that the processing conditions caused marked variations in the nutritive value of

Table VI Nutritive Value of Cottonseed Protein Concentrate in Human Subjects

Protein Source	*Protein Intake*	*% Absorption*	*Biological Value*	*NPU*	*Subjects*
Cottonseed protein	28 86 g /day	81 97	62 12	50 83	Children
Cow s milk	30 55 g /day	90 73	82 15	74 50	Children
Cottonseed flour	5 g /kg	71	57	41	Prematures[b]
Cow s milk	5 g /kg	85	79	67	Prematures

(27 28)
[b] (7*)

different meals Since the free gossypol levels were below the growth-inhibiting level for chicks, the effects could not be attributed to this pigment Processing of cottonseed by hydraulic or screw pressing inactivated most of the gossypol but damaged the quality of the protein This dual nature of the effect of heat on cottonseed protein stimulated re-examination of the nutritional significance of free gossypol and of the nature of the damage to protein quality and resulted in developing processes for reducing or eliminating both disadvantages, as well as increasing the efficiency of oil extraction The prepress solvent extraction method is now in common use (3, 33)

More recently direct solvent extraction has been used A process developed at the Southern Regional Research Laboratories (SRRL) (35, 50) uses a solvent mixture of hexane acetone water, the Vaccarino process (25) uses only acetone

Most attention is now being given to the prepress solvent and direct solvent processes for preparing cottonseed flour for human consumption

The changes in some chemical components taking place in cottonseed meal during screwpress processing are shown in Table VII (19) The extreme conditions and the poor quality of the seed resulted in a product of rather low nutritional qualities as judged by a decrease in available lysine from 3 13 to 2 48 grams per 16 grams of nitrogen In Table VIII are shown the changes in some chemical constituents when cottonseed is processed by the prepress solvent method (19) Fat content decreased while total nitrogen increased with respect to the various operations during processing Free gossypol also decreased significantly, with the greatest change taking place at the expeller level Most of the rats fed with samples obtained before the conditioning of the seed died within 4 days after initiation of study On the other hand, the animals grew normally when fed samples obtained on subsequent operations Weight gain was lower on samples from the solvent extraction tower and from the final product, suggesting a decrease in available lysine during these two operations

Boyer, and Holt (72) evaluated cottonseed protein in premature infants, and showed it to have a net protein utilization of 41% when corrected for nitrogen lost in feces The biological value at protein intakes of 5 and 7 grams per kg of body weight, was 57 and 46%, respectively Cow's milk fed at an intake of 5 grams of protein per kg of body weight had a biological value of 79%

Table V Nutritive Value of Human Grade Cottonseed Protein Concentrate on Several Experimental Animals

	Biological Method			
Diet	*PER (rat) 10%*[a]	*VPU (rat) 10%*[a]	*VBI (dog)*	*Growth (chick) 20%*
Cottonseed protein	2 14	50	0 60	201
Cottonseed protein + lysine	2 40	—	0 72	308
Cottonseed protein + lysine + methionine	—	—	0 85	303
Casein	2 38	72	0 78	—

[a] Protein level

Methods of Processing The industrial methods of removing oil from cottonseed are essentially the same as those for processing other oil containing seeds Because of the demand for better extraction methods, better quality of oil, and better quality meal, important technological modifications have been developed and introduced and newer and more efficient technologies have evolved The details have been described (3, 33) and only conditions directly affecting nutritive value are mentioned in this report The first industrial method for processing cottonseed, hydraulic press processing, is still used in certain underdeveloped areas of the world Although screw press processing is the most common method, treatment of the seed by these two processes is essentially the same The screw press processing method is more efficient for oil extraction than the hydraulic press method, but the latter is less damaging to the protein quality of the seed

Much work on the utilization of cottonseed protein in the form of meal and on its nutritive value was performed using the by-product of cottonseed obtained by these processes It was soon found that the nutritional value of the protein of such products was of very low quality, because of excessive temperature, pressure, and time used in processing Examples of the effect of commercial processing on the nutritive value of the protein of cottonseed meal have been reported by Milligan and Bird (59) using chicks and by Horn *et al* (42) using rats These investigators reported that the processing conditions caused marked variations in the nutritive value of

Table VI Nutritive Value of Cottonseed Protein Concentrate in Human Subjects

Protein Source	*Protein Intake*	*% Absorption*	*Biological Value*	*NPU*	*Subjects*
Cottonseed protein	28 86 g /day	81 97	62 12	50 83	Children
Cow's milk	30 55 g /day	90 73	82 15	74 50	Children
Cottonseed flour	5 g /kg	71	57	41	Prematures[b]
Cow s milk	5 g /kg	85	79	67	Prematures

(27 28)
b (72)

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Most attention is now being given to the prepress solvent and direct solvent processes for preparing cottonseed flour for human consumption

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Table VII Changes in Fat, Nitrogen, Free-Amino Groups of Lysine and Free and Total Gossypol during Cottonseed Processing by Screw Press

Position in Process[a]	Fat, %	Nitrogen, %	Lysine G /16 G N	Gossypol[b] Free, mg /100g	Gossypol[b] Total, G %
Seed	34 4	5 01	3 13	203	1 94
Cooker	28 0	4 34	3 22	193	1 48
Drier	29 6	4 00	3 52	200	1 46
Conditioner	30 4	4 38	3 12	200	1 49
Expeller	5 2	6 22	2 83	43	1 16
Product[c]	4 7	5 33	2 48	46	1 14

[a] Samples taken after operation
[b] On fat free basis
[c] Ground to pass 80 mesh screen

Table VIII Changes in Fat, Nitrogen, Gossypol and Nutritive Value of Cottonseed Processed by Prepress Solvent Extraction

Position in Process	Fat, %	Nitrogen, %	Free Gossypol, Mg /100 G	Mortality, Start/Finish	Wt Gain, G
Seed	33 3	5 60	930	8/1	—
Cooker	32 8	5 29	951	8/1	—
Dryer	33 0	5 71	933	8/1	—
Conditioner	33 0	5 00	872	8/5	−13
Expeller	10 0	7 09	98	8/8	76
Solvent ext	2 8	7 85	64	8/8	58
Flour	3 4	8 28	69	8/8	62

Samples taken after operation

Chemical and biological data for meals prepared by different processing procedures are presented in Table IX It is evident that the prepress solvent-extracted meals are better than the screw-pressed meals, however, the meals prepared by azeotropic solvent extraction were superior to those prepared by the other methods (*57*) A better comparison of meals prepared by azeotropic extraction and conventional meals has been reported by Johnston and Watts (*47*) A further potential advantage is that the mixed solvent extracts aflatoxin from seed with *Aspergillus flavus* (*38*)

The prepress solvent method yields meals only slightly inferior in quality to that prepared by the mixed solvent process In contrast, the screw-pressing method significantly damages the protein quality, as indicated by the lower available lysine and protein efficiency ratios (PER) in chicks (*57*)

Further modifications in present technologies can be introduced to improve nutritional quality In INCAP studies (*19*) carried out in a Central American cottonseed oil mill, several variables were tested, with results shown in Table X Decreasing the load in the expeller and

starting with cottonseed kernels with 18% hulls yielded a product higher in available lysine and lower in free gossypol, after removal of hulls by screening.

Table IX. Evaluation of Cottonseed Meals Prepared by Extraction with Acetone-Hexane-Water Mixture and Other Processes[a]

Process	Gossypol		Available Lysine	Wt. Gain,	PER[b]
	Total	Free	G./16 G. N	G.	Wt. Gain/ Protein
Aceton-hexane-water (106)	0.37	0.035	3.68	290.33	2.19
Prepress solvent (109)	1.04	0.061	3.37	255.00	1.98
Prepress solvent (111)	1.53	0.093	3.13	224.33	1.91
Prepress solvent (112)	0.90	0.036	3.54	249.00	2.01
Screw press (107)	1.20	0.074	2.97	206.33	1.71
Screw press (108)	1.39	0.056	2.50	165.66	1.46
Screw press (110)	1.38	0.075	2.85	180.66	1.61
SBOM	—	—	—	711.33	3.48

[a] (67).
[b] Chicks.
No. of meal.

Gossypol. The extreme conditions used for cottonseed processing have been responsible for the low protein quality of cottonseed flour, but the problem of gossypol has also limited the use of cottonseed protein. This compound present in the cottonseed in small glands is a very reactive polyphenol (3). During processing, the glands are broken and under the heat and pressure, gossypol reacts with free or ε-amino groups of lysine in the protein moiety. Not all gossypol present reacts with lysine since part of it is removed with the oil during pressing and part reacts with other seed constituents. Not all lysine that is unavailable has reacted with gossypol, since carbohydrates are also responsible (68). Binding of lysine by gossypol is, however, an important factor in decreasing the protein quality of cottonseed meal (5, 71). Furthermore, free gossypol is toxic to monogastric animals (3). Therefore, the ideal process for cottonseed would be one which minimizes the inactivation of lysine and reduces or eliminates free gossypol.

Research in this problem is not limited to technologies for conventional seed. Cottonseed varieties have recently been developed which are gland free and therefore, gossypol free. Flours produced from glandless cottonseed have been shown to have high protein quality (47, 70).

Table VII Changes in Fat, Nitrogen, Free Amino Groups of Lysine and Free and Total Gossypol during Cottonseed Processing by Screw Press

Position in Process[a]	*Fat, %*	*Nitrogen, %*	*Lysine G/16 G N*	*Gossypol*[b] *Free, mg/100g*	*Total, G %*
Seed	34 4	5 01	3 13	203	1 94
Cooker	28 0	4 34	3 22	193	1 48
Drier	29 6	4 00	3 52	200	1 46
Conditioner	30 4	4 38	3 12	200	1 49
Expeller	5 2	6 22	2 83	43	1 16
Product[c]	4 7	5 33	2 48	46	1 14

[a] Samples taken after operation
[b] On fat free basis
[c] Ground to pass 80 mesh screen

Table VIII Changes in Fat, Nitrogen, Gossypol and Nutritive Value of Cottonseed Processed by Prepress Solvent Extraction

Position in Process	*Fat, %*	*Nitrogen, %*	*Free Gossypol, Mg/100 G*	*Mortality, Start/Finish*	*Wt Gain, G*
Seed	33 3	5 60	930	8/1	—
Cooker	32 8	5 29	951	8/1	—
Dryer	33 0	5 71	933	8/1	—
Conditioner	33 0	5 00	872	8/5	−13
Expeller	10 0	7 09	98	8/8	76
Solvent ext	2 8	7 85	64	8/8	58
Flour	3 4	8 28	69	8/8	62

[a] Samples taken after operation

Chemical and biological data for meals prepared by different processing procedures are presented in Table IX It is evident that the prepress solvent-extracted meals are better than the screw-pressed meals, however, the meals prepared by azeotropic solvent extraction were superior to those prepared by the other methods (*57*) A better comparison of meals prepared by azeotropic extraction and conventional meals has been reported by Johnston and Watts (*47*) A further potential advantage is that the mixed solvent extracts aflatoxin from seed with *Aspergillus flavus* (*38*)

The prepress solvent method yields meals only slightly inferior in quality to that prepared by the mixed solvent process In contrast, the screw-pressing method significantly damages the protein quality, as indicated by the lower available lysine and protein efficiency ratios (PER) in chicks (*57*)

Further modifications in present technologies can be introduced to improve nutritional quality In INCAP studies (*19*) carried out in a Central American cottonseed oil mill, several variables were tested, with the results shown in Table X Decreasing the load in the expeller and

in lysine when fed at low levels of protein in the diet, children fed the mixture retained as much nitrogen as children fed comparable amounts of protein from milk

VEGETABLE MIXTURE 9 Biological studies with rats, which indicated that the protein from 38% cottonseed flour constitutes the most efficient amount to complement the protein from the cereal grains, gave rise to Vegetable Mixture 9 *(10, 15)* The experimental formula consists of 28% corn flour, 28% sorghum flour, 38% cottonseed flour, 3% torula yeast, and 3% dehydrated leaf meal Mixture 9 contains 27 5% crude protein and has a biological value of 72%

Because the mixture contains 38% cottonseed flour, it was subjected to extensive testing in chicks, rats dogs, and swine The studies included short term protein evaluation tests effects on carcass, liver, bone and blood composition, nitrogen balance experiments, growth and toxicity tests in swine, reproduction and lactation studies in rats as well as multigeneration feeding studies with rats *(13 14 20)* Changes during cooking and the stability and shelf life of the dry product were also investigated The mixture is of good quality free of adverse physiological effects and capable of supplementing diets of poor quality *(42)* At physiological levels of protein intake in children retention of nitrogen was equal to that with equivalent amounts of milk protein *(66)*

Variations of Vegetable Mixture 9 While testing and developing Mixture 9 the several variations shown in Table XI were also studied Mixtures 9 FF and 9 Casein 120 contain 3% fish flour or 3% casein respectively, in place of an equal amount of leaf meal These two derivatives of Mixture 9 are of a slightly higher protein content and nutritive value because of the

Table XI Formulas for INCAP Vegetable Mixture 9 and Variations

Ingredient	*VM9 Exptl* %	*VM9 FF* %	*VM9 Casein* %	*VM9 Commercial Production* %
Maize [b]	28	28	28	29
Sorghum [b]	28	28	28	29
Cottonseed protein	38	38	38	38
Dehydrated leaf meal	3	—	—	—
Fish flour	—	3	—	—
Casein	—	—	3	—
Torula yeast	3	3	3	3
Calcium carbonate	—	—	—	1
Vitamin A I U	—	4500	4500	4500

Uncooked or cooked with and without lime When uncooked or cooked with water 1% $CaCO_3$ is added

[b] Can be replaced totally or partially by any other common cereal grain Calcium hydroxide can be used instead of the carbonate

Table X Changes in Free Gossypol and On Free Epsilon Amino Groups of Lysine During Processing of Cottonseed under Different Conditions

Condition	*Free Gossypol Mg /100 G*	*ε NH-Lysine %*
Full load[a]	91	1 56
75% load[a]	58	1 74
60% load[a]	47	1 79
Kernels without hulls	92	1 71
Kernels with 18% hulls	68	1 84

[a] Amount of cooked kernels fed to expeller

Vegetable Protein-Rich Foods Containing Cottonseed Protein

Cottonseed flour as a protein supplement can play an important role in feeding the malnourished and protein-deficient people of underdeveloped areas and the growing populations of the industrial areas as well A very practical question is whether a protein rich product, such as cottonseed flour, should be made available as such, in the hope that it will be used to supplement the proteins in the basic diet, or whether it should be mixed with some other food so as to give a more complete food For nutritional and practical reasons it seems best to use the protein rich foods in combination with other staple foods to form food mixtures of high protein quality and quantity, which can then serve as protein supplement to basic diets or as complete foods Ideally, these mixtures should be formulated so that the amino acid patterns of the different food components complement each other for maximum nutritional value

The purpose is to obtain a food mixture with an essential amino acid pattern as close as possible to that of the higher quality proteins such as milk, meat, or eggs, otherwise the effectiveness of such protein rich foods is minimized When other nutrients such as vitamins and minerals are deficient, these too should be added to the mixture Such an approach has been used in vegetable protein mixtures developed at INCAP

Formulation VEGETABLE MIXTURE 8 One of the first formulas containing cottonseed flour to receive extensive testing in experimental animals and human subjects was Vegetable Mixture 8 (*11*, *73*), which contained 50% maize flour, 35% sesame flour, 9% cottonseed flour, 3% torula yeast, and 3% dehydrated leaf meal This mixture contains 25% crude protein, with a protein score of 67% based on the FAO amino acid reference pattern (*34*) The amount of cottonseed flour used was small, however, its replacement by skim milk did not significantly improve the protein quality of the mixture Even though the results of biological tests in experimental animals indicated that Vegetable Mixture 8 was deficient

Attention has also been given to mixtures of cottonseed flour and cooked bean flour (*Phaseolus vulgaris*) (9) These two products combine well from the nutritive value point of view when cottonseed provides 70% of the protein and bean flour 30% (Table XIII) Besides increasing the protein value of beans, it significantly increased the total amount of protein From these combinations, Formula 17 was developed, consisting of 35% cottonseed flour, 37% bean flour, 24% cereal grain, 3% yeast, and 1% calcium phosphate, with a protein concentration of about 28% Similar combinations have been prepared with cottonseed flour and other legumes generally with a higher PER than the nutritive value of either of the two components None has yet been fed to human subjects

Table XIII Combination between Dehydrated Black Bean Flour and Cottonseed Protein Concentrate

Black Bean Flour	*Cottonseed Protein %*	*Average Wt Gain, G* *	*PER*[b]
46 7	—	42	1 59
37 3	3 8	55	1 85
28 0	7 6	69	2 15
23 4	9 5	79	2 08
18 7	11 4	92	2 34
9 3	15 2	86	2 32
—	19 0	65	1 94

Average initial weight 42 g
[b] Protein in diets 10%

Chemical and Biological Observations on Food Mixtures Containing Cottonseed Flour

Free gossypol content has been one of the factors limiting the utilization of cottonseed flour Since gossypol is toxic to monogastric animals the significance of its presence in protein rich foods for human feeding becomes of much importance The standard adapted by the WHO/FAO/UNICEF Protein Advisory Committee specifies that edible grade cottonseed flour should not contain more than 0 060% free gossypol This figure is diluted when cottonseed flour is incorporated in food mixtures Still, it was of interest to learn more about the fate of this substance when mixtures are prepared for consumption and after ingestion of the food

Effect of Cooking The results of several cooking experiments with Vegetable Mixture 9 in the presence and absence of calcium and ferrous ions are summarized in Table XIV For these studies, Vegetable Mixture 9 without calcium carbonate was prepared with 38% cottonseed flour, containing from 32 to 96 mg of free gossypol The mixture was cooked as

relatively larger amounts of lysine present in both animal protein concentrates These two mixtures have been tested only in experimental animals

Other variations of Mixture 9 were also studied In this case, the objective was to combine cottonseed protein with cereal grains other than maize, which are more common or used by other population groups All of these variations have been tested in experimental animals and most of them have also been tested in children with good results Mixture 9, made with 58% rice replacing corn, had the highest nutritive value (11, 20)

VEGETABLE MIXTURE 15 Previous results with rats had indicated that mixtures of soybean flour and cottonseed flour, where each provides 50% of the protein, are of higher protein quality than when each ingredient provides 100% of the protein This finding gave rise to Vegetable Mixture 15, which consists of 56% maize flour, 19% cottonseed flour, 19% soybean flour, 3% torula yeast, 1% calcium phosphate, and 4500 I U of vitamin A Mixture 15 contains 27% crude protein and has a biological value of 75% (17)

Other vegetable protein mixtures containing cottonseed flour From food composition data and information of the nutritive value of certain vegetable seeds and cottonseed flour, Bradfield (6), in Peru, formulated two protein supplements, I and II No I consisted of 30% cottonseed flour, 10% quinua flour, 10% *Vicia faba* flour, 10% *Amaranthus caudatus* flour, 2% alfalfa leaf meal, 2% torula yeast, and 35% wheat flour It contained 25 7% crude protein and 352 calories per 100 grams No II contained 35% cottonseed flour, 20% quinua flour, 3% alfalfa leaf meal, 2% torula yeast, and 40% wheat flour The protein and calorie contents were 26 3% and 353 per 100 grams, respectively The nutritive values of these mixtures were found by Graham (39) sufficiently good to consolidate the cure of children with severe protein malnutrition

At INCAP, full-fat peanut flour enriched with cottonseed flour has been tested in experimental animals (12) The data of Table XII indicate that the addition of 10 to 20% cottonseed flour improved the protein value of whole roasted peanuts Addition of peanut flour to supply protein equal in amount to that derived from the levels of cottonseed flour used did not alter the PER

Table XII Supplementation of Full Fat Roasted Peanuts[a] with Cottonseed Protein Concentrate

Supplement Added	*% Added*	*Protein in Diet, %*	*PER*
Peanut protein[b]	10 0	16 2	0 82
Cottonseed protein	10 0	17 6	0 91
Peanut Protein	20 0	20 0	0 89
Cottonseed protein	20 0	24 5	1 10

[a] Amount of full fat peanut in diet 50%
[b] Screw press processed peanut protein concentrate

Fate of Ingested Gossypol To learn more about the fate of gossypol ingested by experimental animals or human subjects fed Mixture 9, studies were carried out to determine if the pigment was present in the feces *(16)* Representative results obtained in dogs (Table XVI) indicate that all total gossypol fed in the mixture appeared in the feces while free gossypol in the feces was about 3 4 times higher than that ingested Since total gossypol includes free gossypol, and good recoveries were obtained for total gossypol in the feces, it would seem that the higher fecal free gossypol was due to liberation of the bound gossypol in the intestinal tract *(16)*

Table XV Effect of Cooking of Vegetable Mixture 9 on Available Lysine and on Nitrogen Balance in Dogs

Addition to VM9[a]	*Available Lysine Mg /100 G*		*Nitrogen Retention % of Intake*[b]	
	Initial[c]	*Final*[d]	*Initial*	*Final*[d]
None	1049	1098	—	—
1% $CaCO_3$ + 0 1% $FeSO_4 \cdot 7H_2O$	1025	1019	—	—
1% $CaCO_3$	—	—	17 5	16 3

[a] VM9 contained 1% starch instead of 1% $CaCO_3$
[b] Initial and final intake of nitrogen 844 and 865 mg /kg /day, respectively
[c] No cooking
[d] 25 min cooking with water

Similar studies were carried out in children fed Mixture 9 Representative results are shown in Table XVII As with dogs, acceptable recoveries in some cases are obtained from total gossypol values, however, free gossypol levels are higher than expected Although these results are not comparable to the dog data, for technical reasons they indicate that the gossypol ingested is being excreted in feces When the children were no longer fed cottonseed flour free and total gossypol disappeared from feces *(44)*

Protein Quality of Cottonseed Flour Samples Prepared by Two Industrial Processes It was desirable, for practical purposes, to find out if flours prepared by modified prepress solvent extraction methods were inferior to flour prepared by azeotropic solvent extraction, which has been reported to yield flours of high quality The two types of flours were used to prepare Mixture 9 and fed to children at two levels of nitrogen intake The results shown in Table XVIII indicate that the protein quality of the two samples is essentially the same at intakes of 2 and 1 grams of protein per kg of body weight per day, and lower than that of skim milk protein only at the lower level of dietary protein Protein digestibility was lower in both

Table XIV Effect of Cooking on Free and Total Gossypol Content of Vegetable Mixture 9 in Absence and Presence of Calcium and Ferrous Ions

Addition to VM9[a]	*GOSSYPOL* Free Mg /100 G *Initial*	*Final*	*% Loss*	Total G /100 G *Initial*	*Final*	*% Loss*
None	22 4	10 8	51 7	0 34	0 35	—
$CaCO_3$	17 9	7 6	57 5	0 41	0 40	—
$Ca(OH)_2$	17 9	5 5	69 2	0 33	0 33	—
$FeSO_4$ 7 H_2O	16 8	10 1	40 0	0 34	0 33	2 9
$FeSO_4$ 7 H_2O pH 9 15	22 1	0	100 0	0 41	0 38	7 3
None pH 9 18	31 5	3 1	90 1	0 43	0 42	2 3
Ca $(OH)_2$ + $FeSO_4$ $7H_2O$	10 9	2 9	73 4	0 31	0 25	19 3

[a] 1% Ca CO_3, 1% Ca $(OH)_2$, 0 1% $FeSO_4$ $7H_2O$

recommended for human consumption and freeze-dried Free gossypol decreased about 52% when the mixture was cooked in water alone The presence of 1% calcium carbonate or calcium hydroxide in the mixture decreased free gossypol level to 57 and 69%, respectively The addition of 0 1% ferrous sulfate ($7H_2O$) and cooking reduced free gossypol only 40%, which is less than the decrease when cooked in water alone The presence of calcium and ferrous ions during cooking caused a decrease of up to 73% Cooking at pH 9 in the majority of cases caused a complete disappearance of free gossypol (*18*) Available lysine content has not been affected The representative results shown in Table XV indicate that even after 20 minutes of cooking Mixture 9 with and without additional compounds, lysine remained essentially the same Nitrogen balance studies provided further evidence that protein quality of the mixture is not impaired by cooking (Table XV)

Cooking is not necessary to decrease free gossypol levels in the mixture, if sufficient calcium and ferrous ions are present Calcium and ferrous ions each protected swine from gossypol toxicity, although not completely When both were present, full protection was achieved The animals gained weight and appeared as well as those fed soybean oil meal (*46*)

The disappearance or destruction of gossypol upon wetting and cooking with calcium and ferrous ions is still not understood A possible explanation is that the gossypol is converted to compounds such as gossypurpurin, which decompose under the conditions of treatment Since available lysine and nutritive value remain essentially the same, it can, therefore, be assumed that the decrease in gossypol occurs in a different manner than when free gossypol is decreased by binding with lysine during processing for oil extraction

It is evident from Table XIX that at protein intake of 2 grams per kg of body weight and above, nitrogen retention of the children is comparable to that with milk protein feeding When protein intake is 1 gram per kg per day or below, nitrogen retention from Mixture 9 is somewhat lower than with milk, because Mixture 9 has a minor deficiency of lysine, which becomes apparent at low protein intakes (*66*, *67*) Although these tests lasted about 3 weeks, children have been fed the mixture for much longer times Representative results of weight changes up to 90 days are shown in Figure 2 All the children gained weight as normally as with milk No adverse effects of any kind were noted A much longer experience is available In an experimental program in a highland Indian village in Guatemala, all children under 5 years of age have been offered a supplement of 27 grams of Incaparina per day (7 5 grams of protein) made up in milk (7 5 grams of protein) Twenty-five children have received the supplement at least two thirds (50% and over) of the possible days for over 4 years, and ten for 5 years No adverse reactions have been reported, and children receiving the supplement have grown and developed well by village standards (*68*)

Table XVIII Nitrogen Balance of Children Fed Skim Milk and Two Cottonseed Flours Prepared by Different Processes

Test Protein	*Intake, Mg /Kg /Day*	*Nitrogen Absorbed, Mg /Kg /Day*	*Nitrogen Retained Mg /Kg /Day*
Skim milk	305	255	82
CSF B	302	218	72
CSF CF21[b]	303	237	77
Skim milk	164	128	51
CSF B	161	109	24
CSF CF21	163	117	18

[a] Cottonseed flour B prepress solvent extracted flour
[b] Cottonseed flour CF21 azeotropic solvent extracted flour

Table XIX Nitrogen Balance of Children Fed Skim Milk and INCAP Mixture 9 at Different Levels of Protein Intake

Protein Intake G /Kg /day	*No of Children*	*No of Balances*	*Milk Nitrogen*		*VM9 Nitrogen*	
			Absorption %	*Retention of Intake*	*Absorption %*	*Retention of Intake*
> 4 0	1	2	84 4	22 0	66 1	24 1
3 0–3 9	4	11	84 9	17 1	70 2	16 8
2 0–2 9	9	48	82 6	16 3	68 9	17 8
1 0–1 9	4	13	78 1	24 9	66 2	15 5
< 1 0	2	2	67 2	8 1	59 1	4 5

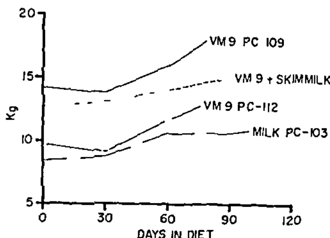


Figure 2 Weight of children fed Mixture 9

Tolerance and Acceptability Vegetable protein mixtures containing cottonseed flour have been tested for acceptability and tolerance by different rural population groups in several Latin American countries In all these trials, the mixtures were highly acceptable, indicating that cottonseed flour is of good nutritive value, palatable, and free of toxic effects Furthermore, these findings indicate acceptability of the food in spite of the color of the product

Other Characteristics of Cottonseed Protein Containing Foods Some of the physical characteristics of food products containing cottonseed flour have been mentioned In others, such as maize or wheat flours supplemented with cottonseed protein concentrate, the principal visual characteristic is the color This is yellow light brown, which upon cooking or baking becomes darker In our experience, color has not caused any serious acceptability problems The color is masked when chocolate is added, as in some acceptability tests in Central America and Mexico

The taste of the foods containing cottonseed flour is either bland or slightly nutty Some flavor concentrates are usually added, such as vanilla, cinnamon, or anise, as well as sugar When sweetened, this food is a filling and satisfying drink

The mixtures for child feeding are usually consumed as gruels A *possible disadvantage is that they become thick, because of the starch from* the cereal grains The problem can be solved by adding from 0 1 to 0 3% of amylolytic enzymes, which liquefy the gruel upon cooking If it is cooked mildly with small amounts of water and allowed to cool, it becomes a gel which can be consumed as a pudding and, with proper flavoring, is *very tasty*

The keeping quality of cereal mixtures containing cottonseed flour is satisfactory, as measured by fat acidity and biological tests with experimental animals INCAP studies showed that the products kept very well

up to 6 months even under tropical conditions, the major problem was infestation by insects If the packaging material allows moisture to penetrate, the mixture eventually becomes darker and nutritive value decreases significantly

The mixtures discussed are now marketed as powder passing an 80-mesh sieve Several types of food preparations could be marketed, such as cakes, cookies, bread, and other baked goods Flavor and structure can be improved by precooking followed by drum drying, however, drying must be carefully controlled to conserve nutritive value Many kinds of food preparations have been made from the mixture They can be used as substitutes for part of the cereal flour or starch in the preparation and cooking of many foods

Other Food Uses

Besides the important use of cottonseed flour in protein-rich foods it is being used as an additive in bakery products, a filler in meat-like food products, and a protein supplement to cereal grain flours

Additive Cottonseed protein in the form of flour is used to prepare a few bakery products such as cookies, crackers, doughnuts, and other foods of a similar nature in amounts varying between 1 and 6% The cottonseed flour reduces dough stickiness, binds water, benefits machining properties reduces fat absorption and increases shelf life because of antioxidant properties Doughnuts absorb 2 to 3% less fat when cottonseed flour is included in the formula Overman (*62*) reported that 3 to 10% cottonseed flour delayed the development of organoleptic rancidity in raw food mixes and to a lesser extent in baked pastry This property has been attributed to the tocopherols present in cottonseed flour, it might also be due to the small amounts of free gossypol present since it is an antioxidant and a polymerization inhibitor (*3*, *4*)

Filler The use of cottonseed flour in ground meat products such as sausages, meat loaves, hamburger patties, frankfurters, and baby meats has not been studied very extensively

Duckworth and Woodham (*29*) have described the preparation and nutritive value of frankfurters made of 34 5% lean beef, 10% lard, and 14% cottonseed flour The raw sausage mixture contained 14 2% protein and was cured for 30 minutes at 85° to 88°C The nutritive value of the sausage was as good as that of sausages made with 14% milk replacing cottonseed flour Furthermore the cottonseed flour frankfurters supplemented cereal diets, this work represents a step forward in utilizing cottonseed flour for this purpose

Protein Supplement Besides serving as a major protein constituent in protein rich foods described above, cottonseed flour can be used as a supplement to the diet and as a supplement to cereal grain flours

Supplement to poor-quality diets The approach of using cottonseed protein as such to supplement diets low in protein quality was first studied by Kuppuswamy *et al* (*54*) in India Their results indicate that 10% cottonseed protein improved the quality of South Indian diets by supplying some of the deficient amino acids as well as additional protein Subrahmanyan *et al* (*74*) and Krishnamurthy *et al* (*52*), using 10% of cottonseed flour prepared from cottonseed kernels extracted with alcohol, investigated its supplementary value to wheat and ragi diets in India Both supplemented diets were significantly better than the unsupplemented ones

Supplement to cereal grain flours Properly prepared cottonseed flour contains protein of high nutritive value and offers an excellent means of supplying dietary proteins to extend and partially replace protein foods of animal origin Cottonseed flour was first used for enriching wheat flour by Jones and Divine (*48*) They found that the addition of as little as 5 parts of cottonseed flour to 95 parts of wheat flour produced mixtures containing 16 to 19% more protein than wheat flour alone and a protein combination superior in its growth-promoting value to isonitrogenous quantities of wheat flour

Womack *et al* (*78*) reported that bread made from 10 parts of cottonseed flour to 100 parts of white wheat flour gave greater gains than equal amounts of bread without cottonseed flour Bread made with 4 parts of nonfat milk solids and 8 parts of cottonseed flour to 100 parts of white wheat flour gave greater gains in weight than equal amounts of bread made with milk solids but without cottonseed flour However, NER's were not significantly different at the 10% protein level Jarquín and Bressani (*45*) also found that addition of 8 to 10% cottonseed flour improved the protein quantity and quality of wheat flour and white wheat flour, as measured by weight gain and protein efficiency ratio (PER) in growing rats The bread made from it was acceptable to rural workers in Guatemala

El Katib (*32*) reported that the protein quantity and quality of maize flour could be improved by adding 4 to 10% cottonseed flour Bressani and Marenco (*22*) described the improvement of lime treated maize flour with several vegetable and animal protein concentrates Significant improvement in weight gain and PER was obtained by adding 10% cottonseed flour to lime-treated maize flour The protein content increased from about 9% in the "masa" flour to about 14% in the enriched product "Tortillas" made from this mixture did not differ greatly in flavor and color from tortillas made from yellow maize

Results of baking experiments using a wholesome cottonseed flour in all types of baked foods were reported by Summers, Mead, and Thurber (*75*) These baked foods were offered for sale over an 18 month period with no evidence of allergies or other disturbances Baked foods with and without added cottonseed flour were equally acceptable

Similar results have been found by Elías *et al* (*31*) for enriching rice flour with protein Although the increase in nutritive value is less than observed with other cereal grains, protein quantity and quality are both improved

These studies indicate that cottonseed flour has a definite place in enriching cereal grains An advantage of this method of enrichment is that the protein-enriched food can be maintained in the common physical form in which it is customarily eaten For example, in Mexico, Central America, and other Latin American countries, maize is consumed in the form of tortillas

Conclusion

Vegetable protein mixtures containing cottonseed protein in the form of cottonseed flour have already found a successful market in several countries in Latin America

Among the countries now producing commercial foods containing cottonseed flour is Guatemala, where current production of Vegetable Mixture 9 (Incaparina) amounts to 120 000 pounds per month Surveys have indicated that there are now a wide knowledge and acceptance of the product and its nutritional value among consumers As high as 90% of the people in most areas of the country hear about Incaparina, and more than 40% of these are using the product either occasionally or regularly In Colombia where Mixture 15 is being produced, commercial production has reached about 200 000 pounds per month after 2 years Recently, Venezuela has started market testing of Formula 9 and the first reports indicate that it should be successful

The extensive research carried out at INCAP on foods containing variable amounts of cottonseed flour has proved that as a human food it is safe nutritious, inexpensive, and palatable The Incaparina program and the increased world production and consumption of foods with cottonseed protein have had a significant impact in many parts of the world, and reports on new technologies for the preparation and use of cottonseed flour for human consumption are being published from India Brazil, Peru the United States, and Guatemala (*25*) Not only are new technologies for better cottonseed flour production being developed, but it is almost certain that cottonseed protein will find many new uses in foods for man in the near future

Summary

Cottonseed protein has been a recognized human food ever since vegetable protein mixtures were developed at INCAP to eradicate protein malnutrition People of low and high economic resources are using such

mixtures as regular supplements to their daily diet Since 1961, 1,083,637 pounds of cottonseed in the form of flour (50% protein) have been produced for human consumption in Latin America Cottonseed protein has also been used for enriching cereal flours, and in preparing noodles, flaked breakfast foods, sausages, and other foods Amounts used vary up to 38% The nutritional value, stability, tolerance, acceptability, and shelf life of these foods have been good The production of cottonseed protein for human use has been limited by inadequate technologies Research has shown, however, that changes in the prepress solvent process yield a product of as good a quality as that from newer technologies Furthermore, in vitro and in vivo studies indicate that moist cooking in the presence of small amounts of calcium and ferrous ions eliminates gossypol toxicity Gossypol is excreted in feces to a very large extent The color of cottonseed flour has met with some objections, but chemical treatment gives a product of a light color with no changes in nutritive quality

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Full-Fat Soybean Flours by Continuous Extrusion Cooking

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Extrusion processing, a method continuing to find new and wider applications in the food industry, is adaptable to the production of full fat soybean flour, and can produce an edible flour of good flavor, oxidative stability, and high nutritional value Experimentally produced flours were evaluated by chemical analyses, biological assays, available lysine content, vitamin assays, organoleptic tests, oxidative-stability storage tests, and clinical testing with infants up to 12 months old The future potential of this process for soybeans seems promising, owing to unique characteristics which lend themselves to modern high production, low-cost techniques that can yield a high-quality product

A deficiency in protein is one of the important dietary problems encountered in many of the developing countries of the world Although animal products are a desirable protein source, their high cost and limited availability restrict their use Consequently, there is a large and growing need for high-quality low-cost plant proteins The protein-deficiency problem is particularly serious with infants and preschool children, especially in tropical and subtropical areas of the world Although powdered milk has been of major importance in feeding programs, its supply is limited, and there is a great need for additional sources of food protein

UNICEF nutritionists have observed that deficiencies in the diet of both children and adults in newly emerging countries are not just from a lack of protein they arise from a shortage of both proteins and calories If calories are limited, proteins are consumed for the necessary energy and, therefore, are not available for body building Full fat soybean flours would serve both purposes because soybeans contain an oil high in calories and because flour from them can be formulated easily with indigenous foods Since these flours would contain all the dehulled soybean,

they represent a concentrated form of food and a most economical source of protein as well For use of the flour in beverages, the natural lecithin in soybeans lends emulsifying properties to give a powder easily and rapidly mixed with water to produce an instant dispersion

Introduction of foods of an unfamiliar type can be expected to take time for acceptance We cannot expect people in need of protein supplementation to like the kind of foods we do Instead, the protein must be incorporated into the traditional foods of these populations For the broadest use of soybean flours, the mild, but characteristic, flavor of soybeans must become acceptable to those not familiar with it Extrusion cooking has good potential for making desirable forms of soybeans economically available to developing countries Extrusion is now widely applied by the American food industry for shaping new products and simplifying production of older ones Some new food areas where extrusion processing is being used are pet foods, breakfast cereals, confections, bakery goods, dough processing, cheese, macaroni, sausage, and the many crispy snack items

Extrusion Cooking of Soybeans

In developing a system for cooking whole-fat soybeans, the objectives are to achieve a cooked soybean flour product with a high nutritional value, to cook the beans adequately to control the growth inhibitors in soybeans, and to produce a bland and palatable product substantially free from unpleasant bitterness or "beany" taste and with adequate shelf life without refrigeration

Studies were previously carried out and reported (*1*, *2*) jointly by our laboratory, UNICEF, and the Wenger Mixer Manufacturing Co on a new method for producing full-fat soybean flour based on extrusion cooking Flours of high nutritive value, stability, and flavor acceptance were produced during the experimental program

The process for full fat extrusion cooking is carried out as shown in Figure 1 The soybeans are first cracked, dehulled, and flaked, although the flaking step is optional The material is then preconditioned in two stages with both direct and indirect steam to add water and heat, mixed, extrusion-cooked, cooled, dried, and milled to a flour

Flakes or grits initially have an average of 9 to 12% moisture and the preconditioner adds additional moisture as steam, bringing the product up to approximately 20% moisture on extrusion Retention time in the preconditioner and mixer is approximately 3 minutes

The extruder-cooker has the configuration shown in Figure 1 It consists of at least three sections separated by back-pressure dies (perforated plates) The unit shown is a production model of that used in our cooperative work with Wenger Throughput is approximately 70 pounds per minute

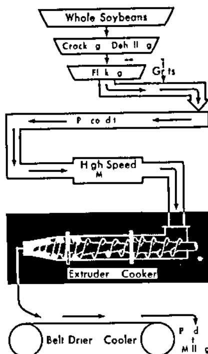


Figure 1 Continuous extrusion cooking process for producing full fat soybean flour

During passage through the various sections, the material is subjected to increased pressure and temperature Pressure is increased by pitched screws separated by the perforated plates that create back pressure and prevent feedback of oil through the feed inlet, which is at atmospheric pressure Finally, the maximum pressure is created in the last tapered-screw or cone-nose section The first two segments are steam-jacketed, but heating is accomplished primarily by mechanical friction of the screw on the material, particularly in the final segment Retention time in the extruder is 1 minute During extrusion, oil is expelled and freed from the cells, but as the material extrudes from the final die plate, the oil is immediately reabsorbed in the meal Freeing the oil from the cells should make it readily available for digestion

Figure 2 shows a time temperature profile of the cooking process

Extrusion-cooking should offer many unique features that will enable it to grow in importance as a process for converting soybeans to food products By this process, the beans are held at elevated temperatures for only a few minutes in contrast to commercial soybean meal toasting at about 212° F for 30 to 45 minutes Thus almost instant cooking is achieved under continuous operation, which results in a minimum of damage to heat-sensitive nutritional factors Temperature of the meal in the final high pressure section reaches values above atmospheric boiling,

however, moisture is not lost, and the extruder section acts as a continuous pressure cooker

Diversified applications, flexibility, and simple control are valuable features of the extrusion process that appeal to the food industry The character of a product can be controlled by altering extrusion temperatures, pressures, or both, to change product viscosity, taste, moisture content, water absorption, and protein solubility Extrusion-cooking of cereal products can pregelatinize starches By incorporating other chemicals one can modify the product chemically on a continuous basis Physical shapes and bulk densities of the product can be controlled by using dies in various forms, shapes, and thicknesses These and other possible variations in processing make extrusion-cooking a highly flexible operation

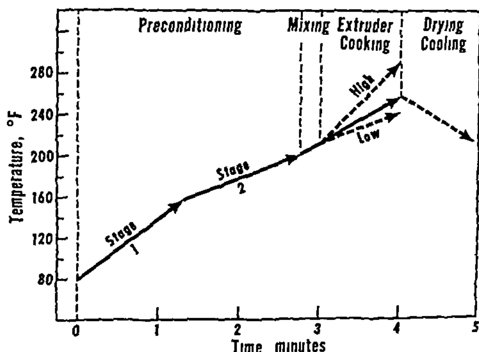


Figure 2 Time temperature profile of extrusion-cooking process

Discussion

Removal of Growth Inhibitors The short cook time with extrusion minimizes damage to nutritional properties but still adequately removes growth inhibitors This removal is demonstrated by low assays for trypsin inhibitor and urease activity Representative products analyze 95% or better destruction of trypsin inhibitor and have low to zero urease activity

Proximate Analyses of Flours The soybean protein contains a good balance of the amino acids essential to human nutrition and, in this respect,

tends to be comparable to animal proteins It is a good source of lysine, more or less lacking in cereal proteins The extrusion cooked products average 5 3% available lysine Table I gives a proximate analysis of extrusion-cooked flours, these values compare favorably with commercial flours

In most cooking processes, the percentage of water-soluble protein, as measured by the nitrogen solubility index (NSI), generally reflects the degree of heat treatment received by the product Extruded flours are cooked to values ranging from 13 to 21 Protein solubility correlates inversely with processing temperature The higher the maximum temperature, the lower the protein solubility

In commercial processing of soybeans, the heat treatment generally reduces their vitamin content Soybeans are outstanding as a source of thiamine, but this vitamin is heat-sensitive and generally heat treatments so necessary to prepare soybeans for animal and human consumption result in destroying thiamine Assays for thiamine, riboflavin, and niacin on the extruded products indicate no significant loss of original vitamin content during processing

Table I Proximate Analysis of Extruded Soybean Flours

Protein %	44
Crude fat %	20
Crude fiber %	<3
Ash %	5
Acid insoluble ash %	0 02
Available lysine % of protein	5 3
Peroxide value meq /1000 g extracted oil	0 40
Free fatty acid % in extracted oil	0 50
Granulation	95+% through 100 mesh

Animal Feeding Tests The results of protein efficiency ratio (PER) tests (*1*) in rat feeding indicate that the experimentally extruded products are of the same general value as commercially prepared full fat and defatted soybean flours

Feeding tests with broiler chicks (*1*) at the University of Maryland for 4 5 weeks showed that for both growth promotion and efficiency of feed conversion the extruded product about equaled a good quality commercial full fat soybean flour and a defatted flour reconstituted with soy bean oil In these tests, 1400 broiler chicks were maintained in floor pens and fed rations containing identical levels of protein and fat from the three products Supplementation with methionine gave about equal improvement in each case

Flavor Evaluation and Stability In organoleptic evaluations (*1*) at the Northern Laboratory by our taste panel, the extrusion-cooked

flours scored relatively high in blandness of flavor, they were neither beany nor rancid They compared most favorably with a commercial soybean flour Accelerated storage tests (1) were conducted at 100° and 113° F up to 9 months At the higher temperatures, good stability was exhibited through the first 9 weeks At 100° F good stability was maintained throughout the entire 9-month period as determined by odor, taste, free fatty acid, and peroxide value test Extrapolation of these data by previous experience to 70° F storage indicates that the extruded flours should have good stability for 1 to 2 years

The Food Quality Laboratory of the ARS Human Nutrition Research Division, U S Department Agriculture, Beltsville, Md , has been studying the use of the extruded full-fat flour in various food products The Beltsville workers report that they are pleased with their preliminary test products, which include biscuits, cookies, noodles, tortillas, yeast bread, and unleavened bread

Clinical Testing with Infants UNICEF has sent approximately a ton of the full-fat flour (1) from the cooperative processing trials to Taiwan, where it has been utilized for acceptability testing, clinically supervised in infant feeding programs at the University of Taiwan In these tests, it was formulated with sugar and supplementary vitamins and fed to children from 4 to 12 months of age in comparison with milk and other soybean products, including an infant food and a conventional full fat soybean product The latest reports (3) from Dr Tung in Taiwan indicated that the extrusion-processed full fat soybean flour supports excellent growth and development in infants as young as 4 months and that these gains are virtually comparable to those observed in babies fed cow's milk

Table II Estimated Processing Costs for Extrusion Cooking Plants Producing Full-Fat Soybean Flour from Dehulled Soybeans

Whole beans processed						
per day, tons	14	30	48	28	60	96
per hour, tons	2	2	2	4	4	4
Shifts of operation per day[a]	1	2	3	1	2	3
Processing costs cents per lb of flour[b]	0 63	0 50	0 45	0 50	0 40	0 36
With classifying[b]	0 76	0 58	0 51	0 63	0 48	0 42

[a] Operation 250 days per year 7 hours production for 1 shift per day 15 hours for 2 shifts 24 hours for 3 shifts
[b] About 88 pounds of flour obtained from 100 pounds of whole beans

Cost Study Preliminary cost estimates have been prepared for the production of full-fat soybean flour by the continuous extrusion cooking method for dehulled soybeans Processing costs are reported in Table II for flour produced by fine grinding the meats obtained from the

extrusion-cooking operation and then classifying the finely ground material, as well as for a system that omits the classifying step Whether classifying is required may well depend upon the specifications of the final product

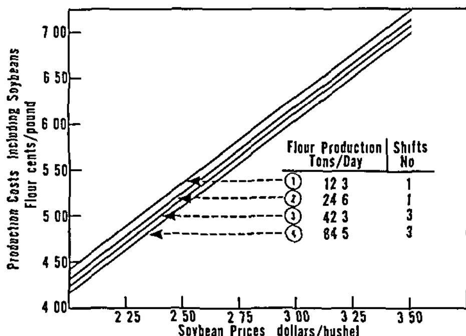


Figure 3 Production costs of full fat soybean flour at various soybean prices

Soybean costs are not included as part of the processing costs but only utilities, labor and supervision, maintenance, and fixed charges

It has been assumed that soybean flour production will be conducted as part of an existing soybean processing plant under such circumstances, such facilities as storage and equipment for unloading, cleaning cracking, and dehulling the beans which are a part of the usual soybean processing plant can be used for flour production The cost of such items, therefore, is not included in the estimated fixed capital investment of a flour plant Estimated fixed capital investment for a plant processing 2 tons of beans per hour is $68,000 if classification is not required for acceptable flour production, if classification is required, the estimated fixed capital investment is $101,000 Estimated fixed capital investments if 4 tons of beans are processed per hour are $107,000 without classification equipment and $173,000 for installations with such equipment Estimated production costs for full fat soybean flour at various soybean prices are given in Figure 3 The curves show that the price of soybeans contributes the greatest share of the flour cost and that relatively small fluctuations in market cost of soybeans can more than equal plant production costs

Conclusion

The extrusion-cooking process can produce a full-fat soybean flour of high nutritive value, mild flavor, and good oxidative stability Growth inhibitors are effectively destroyed while the heat-labile lysine, vitamins, and other nutrients are preserved The process largely removes objectionable taste characteristics, as shown by taste panel acceptance scores Good acceptability and excellent growth development for infants were shown in clinical tests

The cost of soybeans is by far the largest single cost item in the over all process and under some conditions it may be more than 90% of the cost of making full-fat soybean flour The versatility and effectiveness of extruders for rapid continuous cooking should result in their rapid acceptance for processing soybeans into many valuable food products

Acknowledgment

We are indebted to Max Milner of UNICEF, United Nations, New York, for furnishing the reports of Dr Wu and Dr Tung of the University of Taipei, Taiwan, on the clinically supervised acceptability studies Estimates of costs of machinery for the cost study were furnished by Wenger Mixer Manufacturing, Sabetha, Kan , and the Alpine American Corp , Natick, Mass

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Discussion

Oak B Smith* The process reported so well by Mr Mustakas is a short time/high temperature method of continuous extrusion cooking It was developed originally for gelatinizing cereals and was later adapted to cooking mixtures of cereals and oilseed proteins

The period of maximum temperature in this short time/high temperature cook varies from 12 to 17 seconds Prior to this short time cook, steam is applied at atmospheric pressures to a carefully limited degree as a moisturizing agent in a preconditioning zone The temperature

* Wenger Mixer Manufacturing Co , Sabetha, Kan

coefficient which destroys heat labile growth inhibitors is uniquely high, but the cook must be very short in order to involve a minimum of damage to protein quality

The flash pasteurization process produces milk of superior biological feeding value compared to the old batch pasteurization method We believe the brevity of the period at maximum temperatures is no more important for milk proteins than it is for controlling heat labile growth inhibitors in some vegetable proteins

In this light, Wenger decided in 1959 that its continuous cooking process could be adapted to control heat labile growth inhibitors found in soybeans

The first biological evaluation of this method, after about two years of experimentation on our part, was done on rats and on swine, and this evaluation was reported in 1961 by Jimenez *et al* (*2*)

The evaluation reported in the paper just presented by Mr Mustakas was jointly made by the Northern Laboratory, UNICEF, and ourselves, in 1962, as a precursor to clinical evaluation which UNICEF desired to arrange of this cooked full fat soy in Taiwan

A complete report of the Taiwan clinical tests, made by the Medical Faculty of the University of Taiwan, is reported in detail by DeMaeyer (*1*)

With good biological feeding value of this cooked full fat soy product now rather carefully established, we have subsequently turned our attention to cooked mixtures of cereals and oilseed proteins, plus vitamins, minerals and sugars The object here was to produce a sanitary, complete, precooked weaning food and foods for expectant mothers The cereal portions of these foods are completely gelatinized, while the growth inhibitors in soy are simultaneously controlled to produce precooked foods in a variety of forms to meet a variety of needs

These will include

1 Precooked flours to be used in many ways—as atoles as soups, as chapatas, as pasta products, etc

2 As beads or flakes to be used as protein enriched snack items or breakfast cereals

3 As wafers to be used in school lunch feeding programs

4 As protein enriched noodles shell roni, etc

5 Protein enriched bases for purees used as baby foods

6 As soy beverages

Most of the work done to date has been done on soy proteins But work will commence this fall at the Cottonseed Products Laboratory of Texas A & M on control of growth inhibitors in cottonseed under a contract arrangement by UNICEF We ve also done a bit of work on cereal mixtures containing peanut proteins

We believe further that certain of the pulses will prove susceptible to this method of control of growth inhibitors Only a small start has been made at this big job, but we have reason to hope for some reasonable answers to these opportunities for use of pulses indigenous to some of the countries where protein malnutrition exists

Sanitation and shelf life of these cereal mixtures appears to be excellent No insect or larvae will live through the normal process, and bacterial plate counts are very low, with no pathogenes surviving the process

I believe the excellent starts toward commercial production of protein enriched food which have been made by Incaparina and by Quaker Oats on uncooked mixtures and by Hind Brothers in South Africa on cooked mixtures may now be augmented by the impetus given to the protein enrichment of PL 480 commodities by AID and USDA

The exciting news concerning the new high lysine corn reported by Dr Mertz should ultimately prove a big asset to protein malnutrition work everywhere, including mixtures cooked by this method I believe this will be particularly valuable in those areas where soy is not available but where vegetable oilseeds (such as cottonseed and peanuts) which have a deficiency of lysine, are available

I suspect also that protein supplementation with synthetic sources of amino acids as suggested by Dr Howe, will prove of importance in protein malnutrition work, to make up for the deficiencies in amino balance in those malnutrition areas in which the locally available oilseed or pulse proteins are deficient in one or more of the amino acids While the work reported here by Mr Mustakas did not include supplementation by synthetic source of amino acids, DL-methionine was used in one feeding test in the earlier evaluation at Purdue, with some modest gains resulting from that supplementation I anticipate that more important gains by such supplementation will be found in other mixtures of cereals and oilseeds in which lysine is the limiting amino acid

M L Anson Heat processing of soy material is meant to destroy harmful substances and microorganisms without damaging the nutritional value of the bulk protein The desired destructions are due to protein denaturation which has an extraordinarily high temperature coefficient, often denaturation is speeded up hundreds of times for a rise of 10 degrees In contrast, the undesired reactions have normal temperature coefficients, they are speeded up only 2–3 times for a rise of 10 degrees It follows that, contrary to what is often supposed, raising the temperature makes the processing safer *provided* one can reduce the time at the high temperature drastically enough And, of course, reducing the time drastically has the further advantage of increasing the capacity of the equipment

In cooking-extrusion as used in the commercial Wenger machine and in the similar Northern Regional equipment, the material is forced under high pressure through a narrowing cone The high temperature is reached briefly in the cone and the highest even more briefly at the constructed end As soon as the material leaves the cone and the pressure is released the material is cooled rapidly by the flash-off of water hus cooking-extrusion has the great virtue of making possible the high temperature short time processing of even a non liquid, very viscous material such as soybean meal

Because of the temperature coefficient relations which were pointed out the processing conditions which should be chosen are as rapid a through put as is mechanically practical and as high a temperature as this through put permits without damage to the bulk protein

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9

Plant Protein Foods in India

H A B PARPIA and N SUBRAMANIAN

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Oilseed cakes constitute the main untapped sources of protein for human consumption in India and among these, peanut is the largest source Technological improvements in producing edible peanut flour and protein isolate have led to the development of a variety of food products such as the Indian Multipurpose Food (MPF) supplement, Nutro biscuits, vegetable toned milk, spray dried infant food, and high protein weaning food, possessing good consumer acceptance and nutritional value The Indian MPF supplement is being currently produced on a fairly large scale and used in school lunch programs The other products have good scope for commercial exploitation These offer new possibilities of meeting protein needs and improving the nutritional status of the vulnerable groups of the population

In terms of human suffering and the loss of human potential, malnutrition is one of the world's most urgent problems Although this paper discusses primarily the protein foods of plant origin for human consumption, an effort has been made to link the dietary deficiencies of protein with the over-all food problems in the underdeveloped regions of the world, with particular reference to India Due consideration has also been given to the socioeconomic levels of the population

It is estimated that 10 to 15% of the world population is undernourished and up to 50% suffer from hunger, malnutrition, or both (8) Nearly two thirds of the human race live in Asia and Africa, where the food shortage is acute In economically developed countries, generally, the diets are nutritionally more adequate while the developing countries of Asia, Africa and the Far East suffer widespread undernutrition and malnutrition, reflecting both quantitative and qualitative deficiencies Countries of the Far East, or about half of the world population, have only a quarter of the world's total food supplies Europe, North America, and Oceania with only about 29% of the world's population consume 57% of the food The availability of animal foods is also considerably higher in those regions (Table I)

Table I Distribution of World's Population and Food Supplies, by Regions (8) (1957 1959)

Regions	*% of Population*	*% of Food Supplies*		
		Total	*Animal*	*Crops*
Far East (incl China, mainland)	52 9	27 8	18 5	44 2
Near East	4 4	4 2	2 8	5 5
Africa	7 1	4 3	2 8	6 3
Latin America	6 9	6 4	6 7	6 5
Europe (incl USSR)	21 6	34 2	38 4	26 2
North America	6 6	21 8	29 2	10 4
Oceania	0 5	1 3	1 6	0 9
World	100 0	100 0	100 0	100 0

The strikingly low levels of food production per unit of land as well as the low output of foods of animal origin in the less developed areas (Table II) are due to a variety of factors, the more important being small farm holdings, backwardness in agricultural practice and animal husbandry, low soil fertility and the lack of incentive to expand production Losses through infestation and diseases of food crops are also heavy In India rodent and insect pests destroy as much as 20% of the standing crops and 10 to 15% of the stored grains, amounting to more than 20 million tons of food per year There is no doubt that by applying modern science and technology to farming practices, increasing use of fertilizers, and preventing infestation, the availability of food supplies could be increased substantially However in actual practice this task is beset with many difficulties in an underdeveloped socioeconomic situation, and the increase in food production in these areas continues to fall short of expectations

Table II Average Yields of Major Crops and Cattle Products by Groups of Regions (8) (1957 60)

Commodity	*Less Developed Regions*	*Developed Regions*	*World*
Crops 100 kg/ha			
Wheat	9 4	13 3	11 9
Rice paddy	19 1	38 4	19 2
Other cereals	9 0	18 0	13 3
Starches	74 5	122 2	97 1
Pulses	6 1	6 5	6 2
Cattle products			
100 kg /head of cattle	2 6	13 9	6 5

Meat and milk in terms of milk equivalent taking 1 unit of meat as equal to 10 units of milk Averages for 1958–60

Table V Estimated Production and Availability of Legumes for Human Consumption in Different Countries[a] (12)

Country	*Total Production Thousand Metric Tons*	*Grams per Day per Capita*
Argentina	82	8 9
Australia	14	4 7
Austria	5	1 4
Belgium Luxembourg	38	5 2
Canada	66	6 0
Ceylon	4	13 1
Denmark	36	11 2
Egypt	305	24 7
Finland	12	4 6
Germany, Federal Republic	15	4 8
Greece	107	30 1
Honduras	201	30 2
India	10,617	60 8
Ireland	2	2 7
Israel	2 6	8 8
Italy	850	16 2
Netherlands	105	7 7
New Zealand	24	5 2
Norway	1	7 1
Pakistan	900	20 5
Philippines	40	5 3
Portugal	55	17 3
Switzerland	—	5 8
Sweden	38	4 1
Turkey	282	26 3
Union of South Africa	58	5 3
United Kingdom	62	11 0
United States of America	914	10 6
Uruguay	3 7	5 0

[a] Exclusive of peanut and soybean

Food Technological Research Institute, Mysore The introduction of economically and nutritionally important legumes such as the soybean (*Glycine max*) is also being explored in selected areas

Oilseeds Abundant supplies of oilseeds in the protein-deficient countries have led to an increasing realization of their importance as sources of supplementary protein for human feeding India is one of the major oilseed-producing countries in the world and has the largest production of peanut and sesame It also has substantial quantities of cotton seed and coconut While most of the peanut and sesame are processed for oil, over 70% of cottonseed is fed to cattle Coconut is used extensively throughout the country, and about 50% of copra has to be imported to produce oil for edible and industrial uses The production of

meal amounted to over 800,000 tons and the remaining nearly equal quantity of press cake was used locally for livestock feeding and as fertilizer

Much valuable work on peanut processing to upgrade meal quality has been done at the Central Food Technological Research Institute, and a process for preparing a low-fat edible quality peanut flour, free from adventitious toxic factors such as aflatoxins, has been developed The flour has been successfully used to develop a variety of inexpensive nu tritious foods suitable for different groups of the population UNICEF is actively assisting the Government of India to establish two large scale production units for peanut flour The status of technology in respect to peanut protein isolate has also reached the stage of commercial ex ploitation, and this again is due to the pioneering work carried out at the Mysore Institute It has been possible to expand the research program on vegetable proteins with financial assistance from the National Institutes of Health, using the PL 480 funds available in India

Cottonseed The present production of cottonseed in India is about 1 6 million tons, and this could provide 600,000 tons of edible quality flour containing 50% protein if modern methods of processing are adopted A pilot plant with a capacity of 10 tons per day has now been established with UNICEF assistance at the Regional Research Laboratory, Hyderabad, and the Central Food Technological Research Institute is actively collaborating in developing food formulations suitable for child feeding

Sesame The available supply of sesame seed in India is about 500,000 tons, the second largest in the world It is a rich source of methionine and tryptophan and could be advantageously used along with peanut and chick pea flours to prepare protein-rich foods The main obstacle in the extended use of sesame meal in protein blends is, however, the presence of hulls which impart a slight bitterness and dark color to products Much of the calcium in sesame is in the form of calcium oxalate, found in the hulls Recent work at the Central Food Technological Research Institute has shown the possibilities of decuticling the seed by short-period lye treatment Pilot plant studies on the project are now in progress

Coconut India produces 44 8 million coconuts (254,000 metric tons of copra), 64% of which are consumed as such for culinary purposes or as tender coconut The rest is milled for oil, 60% used as human foodstuff and the remaining 40% used to manufacture soaps and cosmetics In addition, India is importing about 85,000 tons of copra The copra cake is not used for human consumption, because of its high fiber content and poor grade of raw material Recently several methods have been developed for processing the ripe coconut kernel instead of copra and utilizing the proteins in human foods (*3*) CFTRI has modified the Krauss-Maffei process in which the ripe coconut kernels are milled and

feeding programs One industrial unit in Coimbatore has begun producing the MPF supplement The West Bengal Government is also starting a plant to manufacture this product for use in its school feeding program

Recent Developments in Protein Foods

Peanut Protein Isolate and Products Based on It The isolation of protein from peanut has been standardized at the institute and recent investigations have shown that it can be used to prepare protein foods for feeding children and also for treating protein malnutrition (*2*) Animal experiments have established that isolation of the peanut protein has not lowered the nutritive value of the protein present in the raw material (*1*) The protein efficiency ratio (PER) of the isolated peanut protein was found to be 1 4 and that of the peanut flour 1 47 This process has now been given to one of the leading industrial concerns in India for exploitation The plant has been set up in Bombay in collaboration with the institute and the National Research Development Corp It is expected to start production early in 1966 It has a capacity of 2 5 tons of protein per day

Milk Substitutes Recent investigations have shown the feasibility of using nutritious milk substitutes made from peanut or soybean to supplement the diets of infants and children One pound of peanut kernel yields 7 to 8 pounds of milk substitute and is palatable to children When fortified with calcium and vitamins, it has a good nutritive value (*9*) The "milk" by itself has a noticeable peanut flavor which meets with some consumer resistance from children and adults, but when subjected to lactic fermentation the peanut flavor is largely masked and the resultant yoghurt is acceptable to the users The institute, for 10 years now, has been manufacturing this product for its staff as well as the general public

Spray-Dried Infant Foods The production of spray-dried milk substitutes for feeding infants is an important feature of the research program of the institute Several blends based on peanut and soybean have been standardized, but major emphasis has been on developing an infant food based on peanut protein isolate and milk (*14*) This blend contains about 22 to 26% protein, 2/3 of which is obtained from peanut and 1/3 from milk The spray-dried powder reconstitutes easily in warm water and can be bottle-fed to infants The composition of the product as well as the flow sheet (Table VIII and Figure 1) for the production are given It has been estimated that the cost of this product on an ex-factory basis will work out to about Rs 4 75 per kg ($ 1 00)

New Infant Food Based on Peanut Flour, Malt Extract, and Buttermilk Solids Infant food described earlier depends on the production of protein isolate by a separate factory Besides, the whey solids from the production of protein isolate from peanut are not utilized To over

Table VIII Chemical Composition of Infant Food Based on Peanut Protein Isolate

(Values per 100 G)

Moisture g	2 8	Vitamin A IU	1500
Protein g	26 2	Vitamin D IU	400
Fat g	18 4	Vitamin E mg	5 0
Minerals g	5 3	Thiamin mg	0 9
Carbohydrates g (by difference)	47 3	Riboflavin mg	1 5
Calcium g	0 95	Niacin mg	6 0
Phosphorus g	0 73	Pyridoxine, mg	3 8

come these disadvantages research is now being concentrated on producing an infant food using peanut flour in place of the protein isolate The main steps in the production of this infant food are

Hydrolysis of gelatinized wheat with barley malt to produce malt extract

Blending of the malt extract along with the enzyme it contains, with cooked peanut flour in order to hydrolyze the starch content of the peanut flour

Extraction of the protein in the peanut by adjusting the pH

Centrifugation and removal of the nonextractable fibrous portions of the peanut

Blending of the centrifugate with skim milk powder or buttermilk powder and hydrogenated vegetable fat

Homogenization

Drying The spray-dried powder is fortified with vitamins by dry mixing (Figure 2)

The composition of the product is given in Table IX The protein content of the product is 25% (54% is from peanut protein, 36% from milk proteins and 10% from cereal proteins) The amino acid composition of the product is given in Table X Animal experiments have shown that the protein efficiency ratio (PER) of this product is 2 5 as such and 2 8 when fortified with methionine (Table XII) Infant feeding trials for this product will be carried out shortly at the Christian Medical College and Hospital Vellore

Studies on the shelf life and packaging of the product are in progress This product will go a long way to stretch the limited milk supplies in India

Foods for Weaned Children In India and other developing countries, infants are weaned from milk to gruels and other starch based foods, very low in protein This results in protein malnutrition, and many cases of kwashiorkor are admitted to the hospitals (*13*) There is an acute milk shortage in all these countries and any foods based on milk or milk protein are normally beyond the reach of the majority It is absolutely necessary, therefore, to develop and manufacture foods for weaned children mainly

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Calcium g	0 95	Niacin mg	6 0
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Centrifugation and removal of the nonextractable fibrous portions of the peanut

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based on vegetable proteins blended in such a way that their amino acids supplement one another

The institute has formulated some precooked foods having a fairly high content of protein which could be fed to weaned infants as gruels or porridge One blend containing peanut flour, green gram flour (*Phaseolus radiatus*), pea flour (*Pisum sativum*), and Bengal gram flour (*Cicer arietinum*), has been standardized The PER of this product when supplemented with methionine is 2 5 as compared with 3 0 of milk (Table XII) The process for preparing this product is shown in Figure 3 It consists

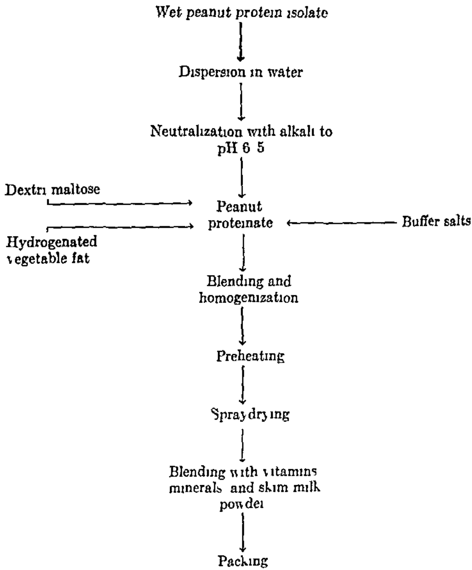


Figure 1 Flowsheet of process for preparation of an infant food based on peanut protein isolate and skim milk powder

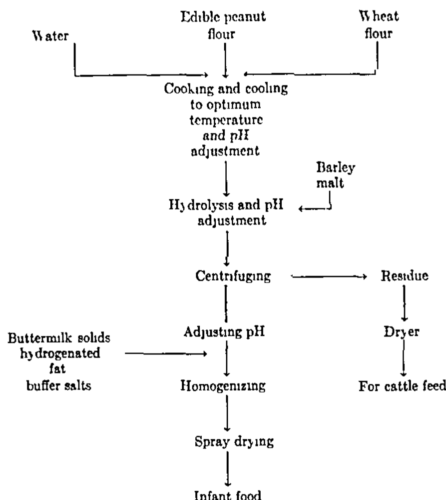


Figure 2 Flowsheet for production of infant food based on peanut flour and buttermilk solids

mainly of cooking the various ingredients, extruding into vermicelli like strands, cutting and flaking

The product has a fairly high acceptability as it has a pleasant taste and can easily be made into a porridge Studies have shown that its shelf life is satisfactory The composition of the product is given in Table XI It is expected to cost about Rs 2 50 ($ 0 50) per kg exfactory when packed in polyethylene bags

Toned Vegetable Milk (Lac Tone) The annual production of milk in India is about 22 million tons and, in spite of the vigorous measures taken by the Dairy Development Department, may not go beyond 25 million tons at the end of the Fourth Plan The reason for this is the low yield of the cattle population and inadequate fodder In India cattle are meant both for milk and draught Hence, adequate stress is not

Table IX Chemical Composition of Infant Food Based on Peanut Flour

(Values per 100 G)

Moisture, g	3 0
Protein, g [a]	25 0
Fat, g	18 0
Minerals, g	5 0
Carbohydrate, g (by diff)	49 0
Calcium, g	1 0
Phosphorus, g	1 0
Iron, mg	6 0
Vitamins (added)	
Vitamin A, IU	1500
Vitamin D, IU	400
Vitamin E, mg	2
Vitamin C, mg	30
Thiamine, mg	0 6
Riboflavin, mg	1 0
Pyridoxin, mg	0 3
Niacin, mg	6 0
Calcium pantothenate, mg	3 8
Vitamin B_{12}, μg	2 2

[a] 13 5 parts of peanut protein 9 0 parts of milk protein and 2 5 parts of cereal protein

laid on milk production Many of the urban milk supply schemes are unable to cope with the demand for milk in the cities The milk supplies are stretched to some extent by producing what is generally known as toned milk—i e , buffalo milk, the fat of which has been toned down to about 3% by mixing with reconstituted skim milk solids

The program, though initially successful in meeting the needs of a larger population, is now faced with the shortage of skim milk powder both through world organizations like UNICEF and in the open market Many of the dairies are unable to meet even the demand for toned milk in the cities To overcome this difficulty, the institute has been experimenting with the use of peanut protein isolate in place of skim milk protein for preparing toned milk The process for making the product is shown in Figure 4 The main steps are reconstitution of the peanut protein isolate at optimum pH in water, addition of liquid glucose to make up total solids, and addition of salts and vitamins for fortification and buffering

This reconstituted emulsion is mixed with buffalo milk, so that the protein content of the blended milk is 3 5%, fat content 3 0% and, the solids not fat 9 0% This blend is homogenized, pasteurized, and bottled The institute produces about 100 liters of this product (Lac -Tone) per day and sells it to the staff members at Rs 0 50 per liter ($ 0 10) It is proposed in the near future to produce this milk in a dairy unit in one

of the large cities The nutritive value of the milk has also been determined by animal experiments and found satisfactory (Table XII)

Relative Costs of Processed Protein Foods Table XIII gives the protein content, ex factory cost estimate, and protein value cost index (PVCI) (*10*) of the protein foods developed in this laboratory Protein foods based on blends of oilseed meals require comparatively little processing and can be manufactured in a relatively simple manner without costly equipment At a cost of Rs 0 05 ($ 0 01) to 0 10 ($ 0 02), a supplement of 20 grams of protein can be provided, but when more processing is involved, as in the case of precooked weaning foods or spray-dried infant food, the cost of production is much higher Though less processing is needed to prepare Lac-Tone its cost is fairly high because protein isolate is used in its production

The consumption of milk-based infant foods has greatly increased recently, and the production is proposed to be raised from 3000 to 6000 tons per annum The production targets under the Fourth Five-Year Plan have been fixed at 25 000 tons per annum There is an acute shortage of this type of infant food in the country at present In India, milk has

Table X Calculated Amino Acid Composition of Infant Food Based on Peanut Flour

(G /16 G nitrogen)

Amino Acids	*Infant Food*	*FAO Reference Pattern*
Tryptophan	1 2	2 4
Threonine	3 5	2 8
Isoleucine	4 4	4 2
Leucine	7 6	4 8
Lysine	5 1	4 2
Methionine	1 6	2 2
Total sulfur acids	3 1	4 2
Phenylalanine	4 7	2 8
Valine	5 4	4 2
Arginine	7 4	—
Histidine	2 2	—

To be fortified with methionine and lysine

Table XI Composition of Weaning Food Based on Peanut Flour and Pulses

(Values per 100 G)

Moisture g	2 7	Riboflavin mg	1 8
Protein g	35 0	Vitamin A IU	3000
Fat g	4 0	Vitamin D IU	300
Calcium g	0 9	Vitamin C mg	30
Thiamin mg	1 1		

Table XIII Relative Cost and Protein Contents of Some Processed Protein Foods

Protein Food	Cost per kg		Protein, %	Cost per 20 g Protein		PER	PVCI[a]
	Indian Rs	US $		Indian paise	US cents		
Fortified peanut flour	1 20	0 22	50 1	5	1 0	1 6	36 4
Indian MPF	1 60	0 32	41 9	8	1 5	1 8	23 6
Infant food (protein isolate and skim milk powder)	4 00	0 80	26 0	31	6 2	2 3	7 5
Weaning food (pulses and peanut flour)	2 50	0 50	34 0	15	3 0	1 8	12 2
+ methionine	2 60	0 52	34 0	15	3 0	2 5	16 3
Infant food (peanut flour and buttermilk solids)	4 50	0 90	25 0	36	7 0	2 5	7 0
Infant food + methionine	4 60	0 92	25 0	36	7 0	2 8	7 6
Lac Tone	0 50	0 10	3 5	30	6 0	2 3	8 1
Buffalo milk	0 85	0 17	3 5	51	10 2	3 0[b]	6 2

[a] $\text{PVCI} = \dfrac{\text{protein per 100 g}}{\text{cost per 100 g in cents}} \times \text{PER}$

[b] PER of sample of buffalo skim milk powder included as control food

Efforts have been concentrated on developing protein foods based on edible grade peanut flour, peanut protein isolate, and legumes with or without inclusion of milk protein The main objective is to fix the ingredients in such a manner as to provide a fairly good balance of amino acids and make the final product of sufficiently high biological value Products have, therefore, been developed with a view to stretching the meager milk supplies and introducing new weaning foods as well as other high protein foods which would have PER equivalent to milk Some of the products have found good consumer acceptance and offer possibility of commercial exploitation in the near future

India has no alternative but to concentrate on low cost vegetable proteins because of the low standard of living, the per capita income

being only $63 per year Due attention has been given to sociological factors in developing the products which would fit into the existing dietary pattern as substitutes and supplements

Protein isolate from peanut flour has been used to treat kwashiorkor in the form of microatomized flour and also as a constituent of processed foods like spray-dried infant food and Lac Tone A new process for using peanut flour in place of protein isolate has been developed, whereby all the fractions of peanut flour excluding the fiber and insoluble carbohydrates are utilized The process does not depend on an external supply of protein isolate and malt extract is utilized in the process as a source of enzyme for hydrolyzing starch as well as to supply some protein Toned vegetable milk using protein isolate has considerable scope in the country Experiments to investigate the possibilities of preparing toned vegetable milk from peanut flour instead of protein isolate are in progress Infant feeding trials on these various foods are being conducted, and animal experiments, already completed, indicate the possibility that these foods could be used successfully

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With modified "dairy-type" equipment the fat recovered is suitable for human consumption

Impulse Rendering Plant Tissues LIPID-PROTEIN ISOLATES Following their success with the impulse process in the gelatin industry, Chayen and Ashworth (4) studied other plant and animal tissues Most plant proteins, unlike bone collagen dissolve in the aqueous phase on impulse rendering, and thus, as would be predicted, plant tissues give more complex end products than bone

Under the conditions prevailing in the impulse renderer these soluble proteins associate with part of the lipid which is also dispersed in the aqueous phase (3) Thus when oilseeds—for example, peanuts—are impulse-rendered in dilute alkali, three fractions are separated by centrifugation free oil, a carbohydrate-rich meal, and a lipid protein complex Most seeds with oil contents greater than about 20% behave according to this pattern Those of lower oil content—for example, soybean—give little or no free oil because most of the oil associates with the protein to form the lipid-protein complex Grasses, alfalfa, and other green leaves give a fiber fraction and a green lipid-protein complex containing the leaf fats and waxes and most of the leaf pigments

Peanuts

As a potential single source of protein for human feeding, the peanut must rank lower than soybeans or cottonseed, whose meats contain more and better protein than the peanut kernel Considered, however, as a potential human food the peanut is preferable by virtue of its blandness and freedom from intrinsic toxins

Manufacture of Peanut Lipid protein Isolate Since 1960, in Plymouth, England, a commercial plant has been worked by International Protein Products Ltd, to produce the lipid protein isolate *Lypro* from peanuts A flow diagram (Figure 1) summarizes the operation of this impulse plant, which has a throughput of 1 ton of raw kernels per hour, and produces *Lypro* together with free oil and a carbohydrate-rich meal

Suitably selected nuts are blanched and washed before impulse rendering in dilute NaOH solution The liquor from the impulse renderer is separated by high-speed centrifugation into three streams carrying carbohydrate, oil emulsion, and lipid protein complex The carbohydrate fraction is washed, dewatered, dried, and ground to a meal The oil emulsion is washed, then heated and centrifuged to give clear peanut oil The lipid-protein complex is precipitated in line, either by pH adjustment or by heating in the presence of divalent cations and is thickened to a paste by centrifugation Finally, the paste is neutralized in line, sterilized, and spray-dried to give the product *Lypro* Table I gives the carbohydrate oil and protein balance obtained in a typical continuous manufacturing run

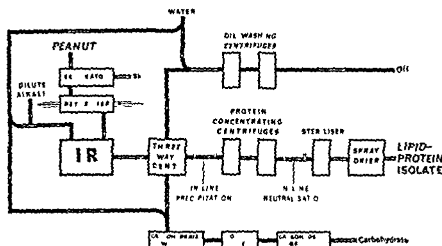


Figure 1 *Diagram of impulse process plant for peanuts*

Carbohydrate Fraction This is a whitish meal containing 18% total lipid and less than 12% protein Several outlets have been proposed for this fraction in the food industry but the whole of our production is now used by the animal foodstuffs industry as a carrier for fats The meal will absorb further lipid—for example as much as half its own weight of tallow—giving a free-flowing product which is conveniently incorporated into high-energy rations (*15*)

Oil Fraction Approximately two thirds of the total lipid contained in the original nuts is recovered as free clear oil with a light yellow color, a faint pleasant odor, and taste of peanuts The free fatty acid content of this oil is exceptionally low (about 0 1%) For many uses—for example, food frying—the impulse process oil may be used directly without further refining, and where a completely odorless and tasteless product is called for this is obtained by conventional mild processing

Lipid protein Isolate *(Lypro)* *Lypro* analyzes as two-thirds protein and one-third total lipid with only traces of carbohydrate ash and moisture It is a bland, cream-colored powder with a faint odor and flavor

Almost four fifths of the original nitrogen of the peanut is recovered as the protein moiety of *Lypro* the remaining one fifth is distributed between the carbohydrate and whey liquor The nitrogen staying in the whey liquor is mainly non protein N A small amount of protein N, equivalent to about 1% of the original nut N can be precipitated from the whey with trichloroacetic acid

NPU AND AMINO ACID COMPOSITION The amino acid composition of the protein moiety of *Lypro* (Table II) resembles published analyses for other protein preparations Determinations of net protein utilization (NPU) give values for *Lypro* in the range 40 to 45 with a digestibility of 95 to 100% this gives a biological value in the range 40 to 47 [All NPU

levels were determined by the method of Miller and Bender (*11*) in the rat, feeding the protein (N × 6 25) as 10% of the diet] Similar determinations made with unprocessed peanut kernels give NPU's within the same range From this it is concluded that in the impulse process there is no deterioration of essential amino acids in the isolation of peanut protein as *Lypro*

The metabolizable energy value for *Lypro* determined by the method of Miller and Payne (*12*) was 5 5 kcal per gram

Table I Composition of Machine Selected Nigerian Peanuts and Fractions Derived by Impulse Rendering

		Composition %	Distribution %
Kernels, 100 parts	Peanut	90	
	Blanching loss	4	
	Skins	3	
	Germs	3	
Peanuts, 90 parts	Crude protein (N × 6 25)	28	100
	Oil	50	100
	Carbohydrate fiber (by difference)	22	100
Lipid protein, 30 parts	Protein (N × 6 25)	65	78
	Oil	32	21
	Carbohydrate	1 5	2
	Ash	1 5	
Carbohydrate 17 parts	Protein (N × 6 25)	11	8
	Oil	18	7
	Carbohydrate fiber	71	70
Oil, 30 parts	Oil	100	66
Protein effluent, 13 parts of total solids ex nuts	Crude protein (N × 6 25) in solution	0 3	14

Total water used for impulse rendering and washing, 12 parts per 1 part nuts

AFLATOXIN In 1961 it was discovered that many batches of peanuts contained a potent extrinsic toxin, aflatoxin This is produced by a strain of the mold *Aspergillus flavus*, which may infect the nuts during growth and storage If infected nuts were impulse processed without preliminary screening and washing, and the *Lypro* was precipitated under acid conditions much of the toxin originally present in the nut was found in the *Lypro* This problem has been solved by rejecting the obviously infected nuts, washing the screened nuts in a fairly strong alkali solution before impulse rendering, and avoiding low pH levels in precipitating the lipid protein complex

Table II Amino Acid Composition of Some Seed Proteins
(G per 16g N)

	Peanut (Lypro)	*Coconut Protein*	*Cottonseed Protein*	*Target Amino Acid Levels (10)*
Lysine	3 0	3 6	3 9	5 3
Methionine	1 1	1 9	1 7	4 2
Cystine	1 4	1 3	1 6	4 2
Leucine	7 6	7 3	6 7	6 4
Isoleucine	3 8	4 8	3 9	5 3
Valine	4 7	6 5	6 0	5 3
Threonine	2 0	3 0	3 6	4 3
Phenylalanine	5 2	5 7	6 3	6 9
Tryptophan	1 0	1 2	1 3	1 0
Histidine	2 5	2 5	2 5	3 5
Arginine	11 6	12 8	7 4	1 8
Total essential amino acids	43 9	50 6	44 9	
NPU	40–45	63	50	92
Digestibility	95–100	95–100	75	
Biological value	40–47	63–65	60	
Aspartic acid	11 8	7 0	9 8	
Serine	4 5	4 8	5 0	
Glumatic acid	17 2	15 9	18 5	
Proline	3 9	2 9	3 4	
Glycine	4 2	4 6	4 7	
Alanine	3 6	4 2	4 8	
Tyrosine	3 7	2 2	3 3	
NH_3	2 1	1 6	1 9	
Total non-essential amino acids	51 0	43 2	51 4	
Total amino acids	94 9	93 8	96 3	

* Phal + tyr

TRYPSIN INHIBITOR In common with many leguminous seeds, the peanut contains a trypsin inhibitor (*1*) In producing *Lypro* about one half of the trypsin inhibitor activity originally in the peanuts is found in the wet lipid protein paste but this residual activity is destroyed in the final stages of sterilization and spray-drying and *Lypro* is free from trypsin inhibitor

ANTIHEMOPHILIC FACTOR There is increasing evidence that hemophilics can achieve hemostasis by eating raw peanuts (*2*) Several patients have found that peanuts can be replaced by an equivalent amount of *Lypro*, which is a convenient source of the factor This suggests that the factor goes mainly into the *Lypro* fraction

to inactivate the gossypol and improve its nutritional value. It was then either dried, or partly dried and, while still hot, squeezed in a normal oil expeller to remove about three quarters of the total oil.

Nutritional Value of Lipid Protein Fraction. Fed to rats at a 10% protein level, these meals gave NPU values of about 50, with a digestibility of 75%, corresponding to a biological value of about 60. These meals have also been successfully fed at a 10% level to laying hens as part of the protein ration.

Table III. Composition of Coconut Kernel and Fractions Derived by Impulse Rendering

		Composition	*Recovery from Dry Kernel*
		%	%
Original coconut kernel	Moisture	43.0	
	Total solid	57.0	
	Protein (N × 6.25)	8.0	
	Lipid	65.0	
	Carbohydrate (by difference)	18.0	
	Fiber	6.0	
	Ash	2.0	
Lipid protein complex	Protein (N × 6.25)	60.0	70.0
	Lipid	35.0	4.0
	Carbohydrate (by difference)	2.5	2.0
	Ash	2.5	
Carbohydrate meal	Protein (N × 6.25)	1.5	4.0
	Lipid	20.0	10.0
	Carbohydrate fiber	75.0	90.0
	Ash	3.3	
Protein effluent	Protein (N × 6.25)	—	25.0
Free oil	Lipid	100.0	80.0
	Free fatty acid	0.28	—
	Color (transmission in 5¼ inch cell) 1.0 Red, 4.5 Yellow		

Leaves

Chibnall (7), in his classic book on protein metabolism in the plant, considered the essential amino acid composition of leaf proteins and concluded that "the excellent nutritive value of leaf proteins is clearly evident." In England there are now two processes for the extraction of protein concentrates from green leaves, that of Pirie (*13*) and the Chayen impulse process. Since 1964 an impulse process unit has been operating under license in Haifa, Israel, for making alfalfa leaf lipid protein. As most

leaves behave in a similar way, it is convenient to describe the results obtained with alfalfa in detail.

Alfalfa

A flow diagram for the impulse processing of alfalfa is given in Figure 3. Fresh alfalfa is fed into the impulse renderer at the rate of 1 ton per hour with dilute alkali. The alkali contains an antioxidant (Santoquin) to prevent oxidative losses of carotenoids during processing. Fiber is removed from the emerging liquor and is washed, dewatered, and dried. The lipid protein complex is precipitated in line by pH adjustment and is thickened to a paste containing up to 50% solids by centrifugation. The paste may be spray-dried, or it may be air-dried, either after extruding to form "noodles" or spreading on a carrier of ground cereal.

Table IV Composition of Undelintered Cottonseed and Fractions Derived by Impulse Rendering

		Composition	*Distribution between Fractions*
		%	%
Original undelintered cottonseed 100 parts	Meats	50	—
	Hull	40	—
	Cotton	10	—
	Protein	23	—
	Oil	18	—
	Fiber	22	—
	Carbohydrate	37	—
	Gossypol (total)	1.1	—
Fractions recovered			
Cotton hull 40 parts	Protein (N × 6.25)	7	15
	Oil	3	7
	Fiber	45	88
	Carbohydrate	45	51
Protein meal, 60 parts	Protein (N × 6.25)	33	85
	Oil	32	93
	Fiber	5	12
	Carbohydrate	30	49
	Gossypol (total)	0.6	
	(free)	0.02	
Squeezed protein meal	Protein (N × 6.25)	43	
	Oil	10	
	Fiber	7	
	Carbohydrate	40	
	Gossypol (free)	0.03	

Table V gives the material balance obtained in a continuous manufacturing run with fresh alfalfa tops

Fiber Fraction About one quarter of the original nitrogen of the leaf remains with the washed fiber fraction Part of this nitrogen is associated with a few leaf cells which escape breakage in the process, but part seems to be associated with the leaf structural tissue elements (5) A disproportionate amount of energy would be needed to liberate this residual nitrogen, which for practical purposes is best utilized by feeding the meal to cattle

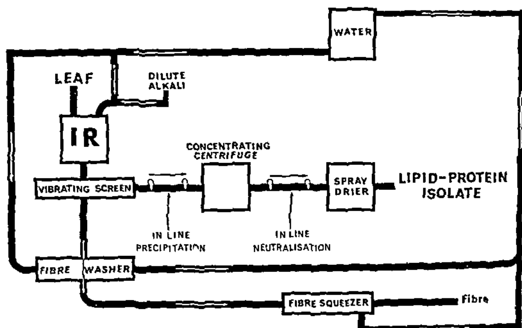


Figure 8 Diagram of impulse process plant for alfalfa

Lipid Protein Isolate More than half of the original nitrogen and most of the lipids and pigments of the leaf are recovered in the lipid-protein fraction The spray-dried product is an intensely green fine powder with a characteristic grassy odor The "noodled" isolates are dried to a granular product which is more convenient to handle

Amino Acid Composition and Biological Value An analysis of the protein moiety of typical alfalfa isolate is given in Table VI

Methionine-cystine is the first limiting amino acid in this protein, giving a chemical score of 62 which agrees with the biological value of 59 (NPU 44, digestibility 74%) found in the rat, and with the biological value of 60 (NPU 60, digestibility 95 to 100%) obtained by feeding an equivalent "synthetic" mixture of L-amino acids

Methionine supplements of 1 5% of protein weight gave optimal effects, increasing the biological value to 72 (NPU 56, digestibility 77%), which compares with the value of 67 obtained by feeding the equivalent L-amino acid mixture

DIGESTIBILITY Only about 75% digestibility is found for leaf proteins in rats (monogastrics), which means that a quarter of the amino acid potential is not utilized This low digestibility is not associated with the processing or drying conditions, because similar values are obtained with freeze-dried complexes and even with undried pastes prepared in the laboratory by mild extraction methods Neither is it associated with the presence of a saponin bloat' factor of the kind found by Cowlishaw *et al* (9) in alfalfa leaf preparations There is no increase in digestibility or NPU after hot water and alcohol washing to remove this putative saponin or on addition of 0 5% of cholesterol to the diet as a steroid competitor From this evidence and from animal feeding studies we conclude that saponin bloat factors are removed in the impulse processing of alfalfa

Fractionation of the lipid protein liquor into a "chloroplastic' fraction and a soluble cytoplasmic fraction showed that the nitrogen was about equally distributed between them When tested in vitro with pepsin and trypsin the digestibility of the chloroplastic fraction was about 60 to 70%, whereas the digestibility of the soluble cytoplasmic fraction was 95 to 100% The amino acid composition of the two fractions is similar (Table VI)

Table V Composition of Alfalfa and Fractions Derived by Impulse Rendering

		Composition	*Yield*
		%	%
Alfalfa 20 parts	Total solid	20 0	
	Crude protein (N × 6 25)	4 4	
	Acetone-solubles	2 0	
Lipid protein 4 parts	Protein (N × 6 25)	61 0	55
	Fiber	0 7	
	Carbohydrates	12 5	
	Acetone-solubles	17 0	
Fiber 10 parts	Protein (N × 6 25)	11 5	25
	Fiber	34 0	
	Carbohydrate	35 0	
	Acetone-solubles	2 0	
Protein effluent 6 parts	Crude protein (N × 6 25)	0 01	20

All other results calculated to dry basis.

Table VI Amino Acid Composition of Proteins
(G per 16g N)

	Alfalfa Leaf Protein	*Alfalfa Leaf Cytoplasmic Fraction*	*Alfalfa Leaf Chloroplast Fraction*	*Target Amino Acid Levels (10)*
Lysine	6.3	6.2	6.5	5.3
Methionine	1.8	1.6	1.8	4.2
Cystine	0.8	1.1	0.9	4.2
Leucine	9.6	7.0	10.3	6.4
Isoleucine	5.3	5.0	5.0	5.3
Valine	5.8	5.7	5.6	5.3
Threonine	5.0	4.9	5.2	4.3
Phenylalanine	6.1	6.5	6.5	6.9
Tryptophan	2.0	1.6	1.2	1.0
Histidine	2.0	3.0	1.9	3.5
Arginine	7.3	8.1	6.6	1.8
Total essential amino acids	52.0	50.7	51.5	
Chemical score	57	65	54	100
NPU	44			92
Digestibility	75	95–100[b]	60–70[b]	
Biological value	59			
Aspartic acid	9.7	9.4	9.1	
Serine	4.5	4.8	4.2	
Glutamic acid	10.6	10.0	10.4	
Proline	4.5	4.4	4.9	
Glycine	4.7	4.8	5.6	
Alanine	5.8	5.7	6.0	
Tyrosine	5.0	4.8	4.9	
NH_3	1.4	1.6	1.6	
Total non-essential amino acid	46.2	45.5	46.7	
Total	98.2	96.2	98.2	

[a] Phal + tyr
[b] Test in vitro

Exhaustive extraction of the chloroplast fraction with polar, nonpolar solvent mixtures, in an attempt to break the lipid protein associations which stabilize the structure of the chloroplast, gave no significant increase in digestibility. We therefore conclude that the incomplete digestion by the nonruminant is associated with fragments of structural elements which are present in the fraction.

Carotenoid Pigments. Provided suitable antioxidants are used, the leaf pigments xanthophyll and carotene are recovered in high yield in the lipid protein fraction.

USES FOR LIPID PROTEIN ISOLATE For animal rations the green lipid-protein isolate may be used, replacing dried alfalfa and other sources of protein This replacement is especially useful in starter rations for animals which cannot tolerate high proportions of fiber Experience has shown that animals are less prone to bloat when the lipid protein isolate replaces dried alfalfa in their ration As a poultry ration component, the alfalfa isolate provides, in addition to protein, carotenoid needed for flesh and egg pigmentation

Pirie (*14*) has advocated using green protein concentrates from leaves for human feeding While it may be possible to conceal small amounts of the green lipid protein complex in certain highly flavored dishes, we feel that for most human feeding purposes the strongly flavored pigments must first be removed This may be done by azeotropically drying the moist paste or by solvent-extracting the air or spray-dried isolate The bland grayish powder thus obtained may then be incorporated as a protein supplement into most traditional 'native" carbohydrate rich dishes The mixture of pigments and lipids obtained from this extraction (Table VII) are separated and the components sold, for example to the pharmaceutical and animal feeding industries

Table VII Analysis of Acetone Solubles from Alfalfa Lipid Protein Isolate

	Mg /Gm	*Mg /Lb*
Copper chlorophyllin	110	50 000
Crude wax	25	11 000
Sterols	10	4 540
Xanthophylls	5 5	2 500
Carotene	3 3	1,500

Other Leaves

We have made lipid protein isolates from many other green leaves including domestic grasses, Bermuda grass maize sugar beet tops and cassava The isolate from cassava is free of cyanide, which is present in high concentration in the original leaf Industrially, Bermuda grass would be processed for the valuable fiber fraction, the small lipid protein fraction could then be isolated as a cheap animal food

In leaf processing it is generally possible to vary the composition of the carbohydrate-fiber and lipid protein fractions by modifying the impulse-processing conditions Thus for example if a fiber fraction of higher protein content were needed for a specific feeding purpose this could be produced by impulse-rendering in weaker alkali solution or even in water

Discussion

The economics of the impulse process for extracting protein foods from vegetable material may be vitally influenced by two considerations, the size of the operation and the ability to utilize all the separated products effectively. For most applications, the optimal size of plant would be one with an impulse unit of 4 tons per hour capacity. The free oil recovered on impulse processing oilseeds is of good quality and may fetch high prices, even without further refining. The carbohydrate-meal fraction is also a valuable product which has many possible uses in modern food technology. Thus, by effectively selling these by-products, the lipid protein isolate may be made more cheaply available for human feeding requirements.

In the extreme example of the coconut, which contains much oil and a little high quality protein, proceeds from the sale of the very high quality oil separated by impulse processing might subsidize recovery of the protein and its distribution to the needy. At present some 3 million tons of coconut are annually converted to copra for oil production, with, as far as human nutrition is concerned, the virtual destruction of the protein present. The oil thus obtained is yellow, and even after refining does not compare in color with that separated in the impulse process.

Commercial evaluation of the pigments, waxes, and fats extracted from leaf lipid protein isolates is now being pursued in Israel and in England. In this fraction lies an enormous potential for the chemical and pharmaceutical industries, and in its proper exploitation may lie the true key to the provision of cheap protein for human feeding.

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I cannot end without thanking M. L. Anson for the unique friendship, help, and encouragement which I have now enjoyed for many years.

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11

Major Seed Proteins and the Concept of Aleurins

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Aleurone grains are subcellular particles of seeds which contain the major proteins "Aleurins" has been suggested as a name to distinguish proteins in aleurone grains from cytoplasmic proteins Solubility properties of the major seed proteins are described, and the solubility classification system is discussed The aleurins of wheat and the globulins of soybeans, peanuts, peas, and hempseed are examined with respect to particle size and association-dissociation properties

The purpose of this paper is to provide background on the properties of the major proteins of seeds, their common characteristics, and their uniqueness, if any

Seeds vary widely in protein content from a low of around 5% to as high as 60% (Table I) Of those with a naturally high protein content, the soybean is perhaps the best example of one widely utilized Even for seeds of lower protein content, such as other oilseeds which contain perhaps 20% protein, the removal of the lipids elevates the protein content of the remainder to over 40% Hence, the nonlipid portion of the seed has a high protein content

Subcellular Location of Major Seed Proteins

One might ask whether there is a special location for most of the protein or is it scattered throughout the seed cytoplasm That seed proteins are in specific locations called aleurone grains has been known for over 100 years In recent years with the advent of electron microscopy, this problem has been investigated anew, and it has been shown definitely that these protein bodies exist and some morphological characteristics have been determined Some idea of the aleurone grains of oilseeds is given in the electron micrograph of a cottonseed section (Figure 1) It is possible

that aleurone grains exist in other seed tissue Bils and Howell (*5*) prepared electron micrographs of germinated soybean cotyledon tissue and observed densely stained bodies, and Varner and Schidlovsky (*24*) observed protein bodies in electron micrographs of germinated pea cotyledon tissue The light micrograph studies made by Duvick (*9*) of the developing corn endosperm showed the presence of protein granules containing mostly zein

Altschul *et al* (*4*) by the use of Carbowax showed that 75% of the proteins of peanuts are in particulates In general, it might be concluded that the major proteins of seeds are in the aleurone grains and these are the unique proteins of seeds Altschul *et al* (*3*) suggested that they be named "aleurins" to distinguish them from the minor proteins of seeds which exist in the cytoplasm It is not suggested that all of the proteins in aleurone grains are the so-called storage or ergastic proteins, which exist primarily for furnishing amino acids or nitrogen for the germinating seeds Enzymes may be associated with them in small quantities But it is suggested that by far the large proportion of the proteins in the aleurone grains are ergastic proteins

Classification and Solubility

Since they are present as a large component of the protein part and are easily crystallized and separated by fractional precipitation, the major

Table I Protein Content of Some Seeds (*1, 23*)

Common Name	*Botanical Name*	*Protein Content*
Cereals		
Wheat	*Triticum aestivum*	12–14
Corn	*Zea mays*	7–9
Rice	*Oryza sativa*	7 5–9
Legumes		
Soybean	*Glycine max*	32–42
Groundnut (peanut)	*Arachis hypogaea*	25–28
Chick pea	*Cicer arietinum*	20–28
Lentil	*Lens esculenta*	23–27
	Onobrychis vulgaris	41
	Tephrosia leiocarpa	44
Oilseeds		
Cottonseed	*Gossypium hirsutum*	17–21
Sesame seed	*Sesamum indicum*	25
Sunflower seed	*Helianthus annus*	27
Walnut	*Juglans regia*	15–21
Others		
Lauraceae	*Avocado* (Persian species)	5
Gramineae	*Fingerhuthia sesleriaeformis*	33
Liliaceae	*Dasylirion wheeleri*	50
Rosacaea	*Rhodotypos tetrapetala*	49

Nitrogen multiplied by 6 25

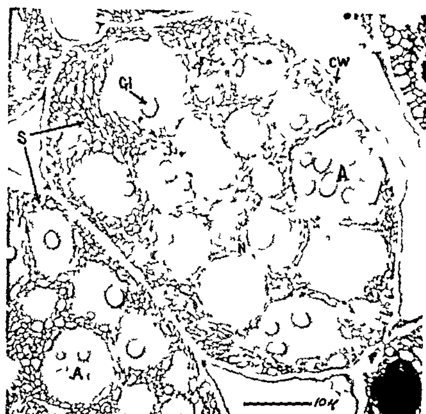


Figure 1 Electron micrograph of cottonseed cotyledon showing aleurone grains

A Aleurone grain
C W Cell wall
Gl Globoid
N Nucleus
S Spherosome

seed proteins have been often considered pure and unique species Indeed, many show one component in the ultracentrifuge and cannot be separated into many components by free electrophoresis This was the state of affairs before the advent of modern techniques of protein separation such as chromatography, gel electrophoresis, and gel filtration

For most seeds, with the exception of many of the cereals, the major proteins are globulins These are soluble in dilute salt solution, are precipitated by moderate salt concentrations and are soluble at alkaline pH and at pH below 4 There existed a nomenclature of globulins unique for each seed edestin from hempseed, arachin and conarachin from peanuts, glycinin from soybean, amandin from almonds and legumin from peas, to mention a few Attempts were made to find common properties of these globulins on the basis of molecular size and amino acid composition, and indeed there are many similarities Table II gives the particle sizes of some major seed proteins They all have large molecular weight, many associate and dissociate into various molecular sizes depending on the pH and ionic

strength of the medium, they are high in arginine and carboxylic amino acids and in amide nitrogen In recent years it has been shown by chromatography and electrophoresis that the classical seed proteins are not monodisperse and some of the properties attributed to these proteins as supposedly unique species must be reconsidered in light of this evidence

The cereal proteins are considered to have two classes of major seed proteins in their storage tissues the alcohol-soluble prolamins and alkali or acid soluble glutelins There are also globulins in the embryo, but since the embryo is such a small proportion of the seed, the corresponding proportion of globulin is usually small

The classification of major seed proteins on the basis of solubility has the advantage of simplicity and is the most universally used one But the questions still remain Are these solely reserve proteins? Are they associated with other reserve material? Are any enzymes associated with the major proteins in the seed? Is there the possibility that some of the proteins are artifacts of grinding the entire tissue in extraction of these fractions? Since the major proteins do not have any obvious biological values, the question of artifact cannot be answered so readily

Table II Particle Size of Some Major Seed Proteins

Protein	*Solvent*	*pH*	*$S_{20,w}$*	*M W* *	*Ref*
α Conarachin	μ = 0 06 phosphate buffer	7 8	12 6	295 000 (estimated)(S)	(11)
	μ = 0 5 phosphate buffer	7 8	7 8	142 000 (estimated)(S)	(11)
Arachin	μ = 0 1	7 9	14 6	330 000(S)	(14)
	μ = 0 1	7 9	9 0	180 000(S)	(14)
Arachin (isolated as aleurin)	μ = 0 06 phosphate buffer	7 8	9 0		(8)
Peanut protein	μ = 0 1 phosphate buffer 7 M urea	8 8	2		(10)
Amandin (almonds)	0 1 M NaCl Tris buffer	8 4		330 000(L)	(1
	6 7 M urea			30 300(S)	(2
Soybean protein, 11 S fraction	μ = 0 5 phosphate buffer + NaCl	7 6	11 8–12 2	363 000(S)	(27
				345 000(L)	(13
Soybean protein 7 S fraction	μ = 0 5 phosphate buffer	7 6	8 0	330 000(S)	(18)
Cottonseed protein	0 5 M NaCl	7 0	9 2	180 000(L)	(19)
γ Gliadin	Aluminum lactate buffer	3 1		50 000(S)	(28)
	4 M guanidine hydrochloride	3 1		26 000(S)	(28)

(S) by sedimentation (L) by light scattering

Aleurins of Wheat

One of the most extensive studies of the protein bodies in seeds is that of the late R K Morton and his colleagues on the developing wheat endosperm Graham *et al* (*12*) showed osmiophilic bodies within a lipoprotein membrane Because of their fragility the bodies were difficult to isolate, but good preparations were obtained which contained about 85% protein Starch gel electrophoresis of the proteins from the isolated bodies showed that they have the patterns resembling the acetic acid–soluble fraction Since they increase in size and number during development and acetic acid–soluble proteins increase rapidly during maturation of the endosperm, there is good evidence that these bodies contain the storage proteins of the wheat endosperm

Globulins

Soybean The mature soybean contains more than 40% protein on a dry weight basis, and the soybean globulins can be isolated by extracting defatted soybean meal with 10% sodium chloride and collecting the fraction which precipitates upon dialysis against water This fraction was originally considered a homogeneous protein and was called glycinin, but it is now evident that glycinin is heterogeneous when examined by ultracentrifugation and by electrophoresis It consists of at least four fractions which have been designated 4, 7, 11, and 15 S on the basis of their sedimentation pattern The 11 S fraction is present to the greatest extent, and the 11 and 7 S components account for 70% of the protein When a concentrated, aqueous solution of soybean protein is cooled, a cold-insoluble fraction is obtained which is primarily 11 S protein

Attempts to understand the relationship between the various fractions observed in the ultracentrifuge are complicated by the fact that the protein can form complexes with other materials in the seed, phytin-protein complexes have been found (*20*) When the phytic acid has been removed by treatment with the anion exchange resin Dowex 1-x10, the solubility of the protein increases, particularly on the acid side of the point of minimum solubility Secondly, the 7 and 11 S components are capable of forming disulfide-linked polymers during manipulation of the protein solutions The disulfide interchange reaction can be eliminated by adding mercaptoethanol routinely to the pH 7 6 buffer containing added NaCl This gives the simplest ultracentrifuge patterns because association is suppressed by high ionic strength and because disulfide polymerization is prevented by the reducing conditions The so-called cold insoluble fraction—the 11 S fraction—was purified to the extent of about 90% by extraction of the acid-precipitated protein with sodium chloride of graded ionic strengths A particle weight of 363 000 by sedimentation diffusion and 345,000 by light scattering was obtained, this compares to the reported

molecular weight of 380 000 for the 11 S fraction obtained by ammonium sulfate fractionation (*26*) Further purification was achieved by Hasegawa *et al* (*18 16*) by means of Sephadex G-200 The impurity was the 7 S fraction and this could be removed by chromatography on calcium phosphate gel The purified 11 S component is homogeneous by sedimentation analysis, but no information is available on its purity by gel electrophoresis

Roberts and Briggs (*18*) purified the 7 S component by gel filtration on Sephadex G-100 It contains only a trace of the 11 S component The 7 S component will associate when the electrolyte concentration is lowered from 0 5 to 0 1 ionic strength to give a 9 S form Both forms have a concentration dependence of molecular weight, extrapolated to zero concentration the molecular weight of the 9 S component is 650,000 and of the 7 S is 330 000 The amino acid composition of the 7 S component is somewhat comparable to the whole globulin but the 7 S component has 5 9% carbohydrate compared with 1 5% for a relatively pure 11 S component Two moles each of serine and glutamic acid and one mole each of glycine, valine and leucine are the *N* terminal amino acids per 300 000 molecular weight This would indicate a minimum of seven peptide chains Both the 7 S and 11 S components will associate, depending on the ionic strength, but there is no interconversion of the 11 S to the 7 S component once the 11 S component has been obtained pure

There are no data to show whether the glycinin fraction is within particles in the soybean cotyledon and might properly be called an aleurin That the proteins in cotyledons are in subcellular particles was suggested by Bils and Howell (*5*) who studied the changes in the developing soybean cotyledon The protein particles are fragile and do not survive solvents which are satisfactory for the isolation of mitochondria, microsomes, and chloroplasts

Peanuts About 90% of the proteins of peanut cotyledons are soluble in a dilute neutral buffer or 5% sodium chloride solution The classical fractionation of the salt-soluble protein is by precipitation with ammonium sulfate That fraction precipitating at 40% saturation was called "arachin" and the fraction precipitating in the range of 40 to 85% was called conarachin Neither of these fractions is pure as noted from its ultracentrifuge pattern Their solutions give complex patterns on diethylaminoethylcellulose (*6*) and many components by polyacrylamide gel electrophoresis (*11*) The major protein of the conarachin fraction was isolated by repeated chromatography on DEAE cellulose until homogeneous by chromatography, sedimentation, and almost pure (about 85%) by electrophoresis on polyacrylamide gel This fraction, named alpha-conarachin, associates and dissociates on the basis of ionic strength of the medium at pH 7 9 At an ionic strength of 0 03 it has a sedimentation coefficient of 13 1 S, at an ionic strength of 0 5 it has a sedimentation coefficient of 7 8 S These give

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the approximate particle weights of 295,000 at low ionic strength and 142,000 at high ionic strength

Dieckert *et al* (7) homogenized cotyledonary tissue of the peanut in nonaqueous medium to prevent the rupture of protein bodies and isolated two fractions which contain 11 and 13% nitrogen, respectively Although these fractions have similar protein composition as seen by chromatography on diethylaminoethylcellulose, one of them contains practically all the phytic acid Electron micrographs of the isolated protein bodies are similar in appearance to those of sections of the tissue itself (2)

The protein bodies of the peanut are fragile and dissolve in 0 5*M* sucrose in buffer at pH 7 0 When unbuffered 0 25*M* sucrose was used one could isolate the protein body fraction Protein bodies were obtained by macerating the peanut cotyledon in 0 05*M* citrate buffer at pH 5, and 0 25*M* in sucrose and 0 05*M* in sodium (ethylenedinitrilo) tetraacetic acid (3) A second particulate fraction was obtained by exposing the lighter particle to pH 6 for 18 hours at 4° C The two fractions differed in their original patterns on diethylaminoethylcellulose and in their lipid content (the heavy fraction contained 8% lipid, whereas the pH 6 stable particle had 45% lipid), both particulate fractions contained 56% protein

The protein obtained by this particulate method of separation was identical with the arachin fraction, except that the arachin ordinarily obtained by ammonium sulfate fractionation was the dimer, this particular method of isolation produced predominantly the monomer Recently Tombs suggested that arachin exists in two variant forms (22)

The classical arachin fraction can be classified as an aleurin The presence of two aleurin fractions might suggest that there are at least two different kinds of particles and several aleurins in the peanut Alpha conarachin, which is a major fraction of the peanut proteins and comprises approximately 25 to 30% of the total fraction, has not been isolated in particulate fractions

Pea Protein It has been known for some time that the major pea proteins are globulins Varner and Schidlovsky (24) observed a large number of spherical bodies averaging 2 microns in diameter scattered throughout the cytoplasm of pea cotyledon in the early stages of germination They designated these as protein bodies and observed that at various stages of development of the pea, the appearance of these bodies follows the course of globulin synthesis

Hempseed Edestin, the protein of hempseed (*Cannabis sativus*), was one of the first proteins to be isolated, since it is easily crystallized Edestin is prepared by extracting defatted hempseed flour at 40° to 50° C and then cooling the extract to 0° C It has long been considered homogeneous, but obviously it is no purer than any of the other proteins mentioned

One recent approach to its purification was by Stockwell *et al* (21), who chromatographed edestin on DEAE cellulose at 50° C and were able

to obtain a fraction which shows an elution pattern of one peak upon rechromatography At relatively high ionic strength this protein has a sedimentation value of 13 4 S, this would approximate to a particle weight of 300,000 The amino acid composition of edestin is given in Table III

Table III Amino Acid Composition of Edestin

(G of anhydro acid per 100 g of protein)

Aspartic acid	11 02(*21*)	11 43(*15*)
Threonine	2 81	3 15
Serine	4 41	4 85
Glutamic acid	14 19	18 67
Proline	3 31	3 19
Glycine	3 33	3 81
Alanine	3 51	4 10
1/2 cystine	0 86	1 04
Valine	3 87	5 40
Methionine	1 60	2 14
Isoleucine	2 88	4 74
Leucine	4 78	6 78
Tyrosine	3 28	4 38
Tryptophan	1 46	1 12
Phenylalanine	3 97	5 64
Lysine	2 18	2 54
Histidine	2 57	2 54
Ammonia	2 54	2 53 (amide N)
Arginine	14 29	15 48
Total	86 9	101 0

Another approach to purification was by Dlouha *et al* (*8*), who purified edestin by chromatography on diethylaminoethylcellulose in 6*M* urea at pH 6 9 Their purified sample showed one elution peak by this technique and gave a molecular weight of 300,000 They were able to effect further purification of this material by reductive cleavage according to the method of Swan which yields *S*-sulfoedestin It gave two spots by electrophoresis on starch gel in urea buffer at pH 8 7 One component (A) moved to the anode and the other (B) to the cathode The two components were separated by chromatography on DEAE cellulose in urea-phosphate buffer of pH 9 A high content of arginine, glutamic acid and aspartic acid is characteristic of component A, much less of these are in component B Neither contains any sugars On the basis of sedimentation they obtained molecular weights of 27 000 for A and 23 000 for B The A chain does not yield an *N*-terminal group and the B chain yields one mole of *N* terminal glycine per mole of protein These authors believe that the edestin molecule is a unit of 300,000 molecular weight which in turn is composed of six subunits, each of 50 000 molecular weight Each subunit in turn is com

posed of two polypeptide chains of molecular weights approximately 27,000 and 23,000 and connected by disulfide bonds

Typical Major Seed Proteins

Eventually, it should be possible to relate proteins to certain biochemical functions and to classify proteins of seeds on the basis of their function The first approach in that direction is to classify seed proteins on the basis of their association with subcellular structures and to divide the seed proteins into two classes those within protein bodies, which can be separated as particulates, and those in the cytoplasmic matrix At the moment, only a few proteins have definitely been shown to be aleurins If for the sake of simplification, we equate the major proteins of seeds to the aleurins and take whatever information is now available about aleurins, we can present two prototypes of proteins that would represent the aleurins of seeds and their major proteins One of these is the major protein of cereals as typified by gliadin of wheat and zein of corn The other would be like the arachin of peanut or any of the major proteins of seeds other than the cereals

No pure major protein has been isolated—that is, no seed protein has been isolated which shows homogeneity by three criteria simultaneously

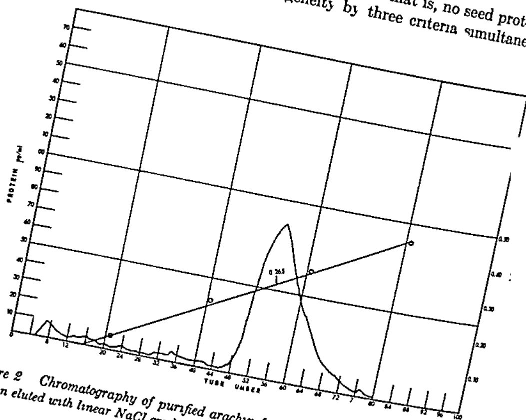


Figure 2 Chromatography of purified arachin fraction on diethylaminoethylcellulose Protein eluted with linear NaCl gradient 0 to 0.6 M in phosphate buffer pH 7.9 μ = 0.03

ultracentrifugation gel electrophoresis and chromatography on ion exchange materials It may be that because of association-dissociation it is impossible to isolate a single molecular size However, we are not of the opinion that this is yet impossible of achievement and have been pursuing approaches involving preparative electrophoresis on polyacrylamide gel In our laboratory we have been able by means of preparative gel electro phoresis to isolate a fraction from arachin which shows only one band on gel electrophoresis and one major component and one very small component in the ultracentrifuge The preparation was homogeneous on diethyl-aminoethylcellulose chromatography, as shown in Figure 2

In view of the absence of complete purity, one can only take a tentative position about the properties of the aleurins, subject to revision when more successful fractionation techniques are developed Until then we can make only general statements about amino acid composition molecular size, solubility, and the property of association and dissociation

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12

Measurement of the Nutritional Availability of Amino Acids in Foods

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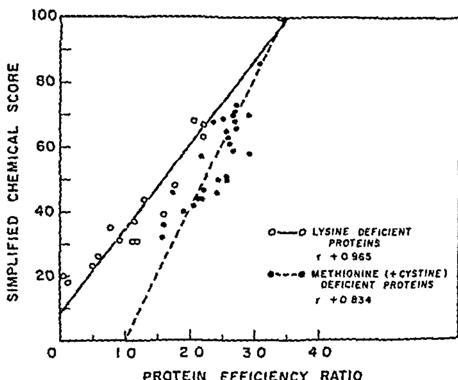
Nutrition Division, Food and Drug Research Laboratories, Department of National Health and Welfare, Ottawa, Canada

The amounts of amino acids available to the body after ingestion of food proteins may be much less than indicated by the amino acid composition of the dietary protein. Chemical score data, based on amino acid composition, may therefore provide a misleading estimate of the value of processed food in ameliorating protein deficiency. Although a variety of chemical and biological procedures have been developed to evaluate amino acid availability, there is a continuing need for improved rapid procedures. A rapid chemical method for determining availability of the sulfur amino acids is particularly needed, since these amino acids often are limiting factors in human diets.

The classical investigations of Osborne and Mendel (*58*) led to a realization that the nutritional value of proteins depends upon their content of the amino acids required for essential metabolic functions. It is not surprising, therefore, that in general the nutritive value of proteins can be predicted with reasonable accuracy from knowledge of their amino acid composition. This fundamental relationship between nutritive value and amino acid composition has provided the basis for a number of methods of evaluating proteins in foods. For example, Block and Mitchell (*3*) developed a procedure for determining the chemical score of proteins, based on their amino acid composition relative to egg protein, which is almost completely utilized by human subjects or the growing rat. A good correlation was found between the chemical score and nutritive value determined in animal studies.

More recently McLaughlan *et al.* (*40*) found a high degree of correlation between simplified chemical score and protein efficiency ratio (PER) values for 43 foods deficient in either lysine or the sulfur amino acids (Figures 1 and 2). In studies on Canadian meals, Morrison (*51*) observed that

prediction of per cent net dietary protein calories from simplified chemical score data gave values which agreed closely with those determined experimentally, if a prediction equation was used which takes into account the nature of the relationship between net protein utilization (NPU) and per cent protein calories These data, summarized in Table I, indicate that the amino acids in the meals tested were available to the body



Courtesy Canadian Journal Biochemistry

Figure 1 Correlation between SCS and PER for 48 foods

Foods apparently deficient in lysine plotted separately from those apparently deficient in methionine (plus cystine)

Miller and Donoso (*47*) concluded that in mixed human diets the sulfur-containing amino acids often are limiting factors Miller and Naismith (*48*) showed that the net dietary protein value of various human diets could be estimated accurately from their sulfur content In subsequent studies, Miller and Donoso (*47*) proposed a chemical method for measuring nutritive value, based on the sulfur and nitrogen content of foods They concluded that for practical purposes the protein scores of mixed diets are equal to 1000 S/N and calculated net dietary protein values from the following equation

$$\text{ND p Cals \%} = \text{protein score} \times P \frac{(54 - P)}{(54 - Pm)}$$

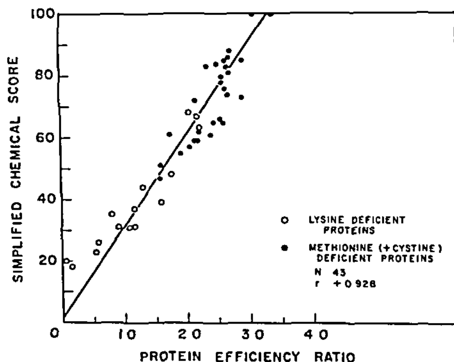
where ND p Cals % = % net dietary protein calories

P = % protein calories

Pm = 400/protein score

The validity of Miller and Donoso's prediction equation is based upon a number of assumptions, one of which is that the amino acids in foods are available to the body That this assumption may not always be valid is indicated by the studies of Miller and Carpenter (*45*) who reported that for a series of seven fish, meat, and whale meals, there was no correlation between the sulfur content and net protein utilization for rats, although the quality of the protein was limited by the sulfur amino acids Cystine and methionine accounted for less than two thirds of the sulfur found in five of the seven samples The digestibility of sulfur ranged from 53 to 90% and the product of sulfur amino acid content and sulfur digestibility correlated closely ($r = 0.93$) with values for NPU

The studies of Miller and Carpenter (*45*) illustrate a principle which is becoming increasingly apparent—that the nutritional value of processed proteins cannot be predicted accurately from data on amino acid composi-



Courtesy Canadian Journal Biochemistry

Figure 2 Correlation between SCS and PER for 43 foods after adjustment of (methionine plus cystine) scores

tion It now is well established that, during processing, the amino acids of foods may be made partially unavailable to the body, with the result that the amounts of amino acids available for metabolic functions may represent only a small percentage of those present in the food protein Continued population pressure on conventional food resources indicates that man will be forced to make ever-increasing use of highly processed protein foods, many of which now are used largely for fertilizer or animal feeds In preparing such foods, careful attention must be paid to factors which influence the availability of the amino acids It is not surprising therefore, that many investigators have devoted considerable time and effort to develop procedures for measuring amino acid availability This report summarizes available information on this important subject No attempt has been made to provide an encyclopedic coverage of the literature The scope of the paper has been restricted to a discussion of recent papers which illustrate important principles

Table I Prediction of ND p Cals % from Chemical Score Data for Canadian Meals

Meal	*Protein Cal %*	*Simplified Chemical Score*	*Limiting Amino Acids*	*ND p Cals %* Found	*ND p Cals %* Calculated *M*[a]	*ND p Cals %* *M & P*[b]
1	16 0	52	L	5 8	5 2	6 8
2	16 1	80	M + C	8 4	8 1	9 9
3	21 4	79	M + C	9 0	9 1	11 3
4	18 7	60	M + C	6 9	7 2	8 4
5	18 1	83	L	8 3	8 8	11 0
6	22 6	72	Tr	8 4	8 8	10 6

[a] *(61)*
[b] *(49)*

Evaluation of Amino Acid Availability

Chemical Methods Perhaps the most useful chemical method developed thus far to measure amino acid availability is the procedure of Carpenter and Ellinger (7), which employs Sanger's reagent *(61)* It is based on the reaction of dinitrofluorobenzene (DNFB) with free amino groups in proteins The ϵ-dinitrophenyllysine (ϵ-DNP lysine) released after subsequent hydrolysis is measured spectrophotometrically

This simple procedure proved useful for estimating available lysine in a range of animal materials (6), but was affected significantly by interference from α-dinitrophenylarginine when applied to autolyzed materials and from dinitrophenol arising from the breakdown of excess DNFB if carbohydrates were present *(10)* Furthermore, vegetable foods may give

rise to colors which interfere with the procedure This interference was avoided by a modification (4) in which methoxycarbonyl chloride was used, but treatment with this reagent led to the unexpected development of a colored histidine derivative (8)

Conkerton and Frampton (10) took advantage of differences in the absorbance of dinitrophenol at 360 mμ in acid and alkaline media to correct for the dinitrophenol present in protein hydrolyzates Other yellow substances such as dinitroaniline also may occur in the hydrolyzates of some dinitrophenylated proteins and introduce errors in colorimetric estimation of available lysine (21) The water soluble imidazole DNP histidine and O DNP tyrosine which are colorless but absorb slightly at 360 mμ are also likely to be found in dinitrophenylated protein hydrolyzates In addition the brown humin pigments that occur in acid hydrolyzates of proteins may contribute to the error in estimating available lysine by the DNFB procedure

Interference in the DNFB procedure from a colored histidine derivative was reduced by a modification of the method (6) which involved removal with diethyl ether of the lysine–methoxycarbonyl chloride reaction product, and use of the resultant blank' value to correct for interference due to histidine Suitable corrections were applied to allow for losses of DNP-lysine during the acid hydrolysis, by making use of the recovery of an internal standard of DNP-lysine added to the samples prior to hydrolysis Handwerck *et al* (21) observed that the conditions of the chemical procedure partly protected DNP-lysine from the destructive effect of acid hydrolysis in the presence of sugars The protection was less effective with starch in the presence of which the recovery of added DNP-lysine was only 74% compared with a mean recovery of 92% obtained with animal products essentially free of carbohydrates (6)

In an attempt to overcome errors in the colorimetric estimation caused by interfering materials Baliga *et al* (2) resorted to a chromatographic separation of ε DNP-lysine followed by colorimetric estimation The ε DNP lysine was separated from the other water-soluble DNP amino acids on filter paper using butanol–acetic acid water (4 1 1) for irrigation The ε DNP lysine which separates as a heavy band with an R_f value of 0 6 was eluted with 1*N* HCl the color density determined at 363 mμ, and the value read from a standard curve The reaction with DNFB was carried out at 40°C as recommended by Sanger (61) Attempts to use higher temperatures in hope of shortening the reaction time gave lower values, probably due to slow destruction of the DNP derivative

Raghavendar Rao *et al* (60) successfully used ion-exchange resin chromatography for separating ε DNP lysine quantitatively from other yellow and brown components in protein hydrolyzates The column was developed with a mixture of methyl ethyl ketone and 3*N* aqueous HCl Calculations of available lysine were made from measurement of absorbance

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at 435 mμ The authors claim that the method lends itself to routine analysis, since one operator can carry out eight determinations simultaneously

Carpenter's procedure using Sanger's reagent has been widely used by various workers for estimating available lysine in vegetable and animal products There is a need for further modifications of the procedure for use with foods rich in carbohydrates to reduce their destructive effect on DNP-lysine during acid hydrolysis

More recently, Mauron and Bujard (*41*) suggested guanidation as an alternative approach for determining available lysine in foods Guanidation with o-methylisourea transforms lysine molecules with a free ε amino group into homoarginine, which is stable during acid hydrolysis The sample containing the protein is made to react with o methylisourea, and the guanidinated proteins are precipitated with trichloroacetic acid and hydrolyzed with 6*N* HCl The homoarginine present then is estimated by column chromatography In lyophilized and spray-dried skim milk powder, about 95% of total lysine was converted to homoarginine and was therefore considered "available" In peanut flour, the homoarginine value was slightly lower than the corresponding ε-DNP-lysine value, but in soybean flour it was higher From the limited data reported by Mauron, it is difficult to appraise the method fully, except to indicate that it appears promising

Moran *et al* (*50*) used the reaction of the dye Orange G with basic amino acids (*16*) to determine the availability of free amino, imidazole, and guanidyl groups of proteins They noted that the dye-binding capacities of soybean meals heated for varying lengths of time were closely related to the growth of chicks fed the meals Ascarelli and Gestetner (*1*) also found that the dye-binding capacity of soybean meal was a good measure of its biological value In other studies, however, we could find no correlation between the ability of Canadian meals to bind Orange G and the nutritional value of the protein contained therein, as determined in the growing rat (*51*)

Suitable chemical procedures to evaluate the availability of sulfur amino acids have not been developed, although the need for them is obvious It is possible that advantage may be taken of the reaction between CNBr and the free terminal methionine residues in a peptide chain (*18*) Cyanogen bromide can cleave methionine peptide bonds in proteins, producing cyanosulfonium derivatives of methionine, which under acidic conditions yield homoserine lactone This substance can be determined colorimetrically It is conceivable these reactions may serve as the basis for a rapid chemical procedure for determining available methionine

Enzymatic Methods In vitro enzymatic studies have demonstrated clearly that amino acid availability and amino acid content of foods may differ markedly Comparative measurements of the enzymatic and

chemical liberation of a number of amino acids have been made by numerous investigators including Evans and Butts (*14*) using soybean protein and Hankes *et al* (*22*) using casein Evans and Butts (*14*) demonstrated that autoclaving soybean protein with sucrose for 4 hours did not seriously affect the in vitro enzymatic release of phenylalanine, threonine, leucine, isoleucine and valine Under the same test conditions, however, 84% of the lysine and 41% of the methionine were not released by enzymatic digestion of the protein sucrose mixture Approximately half of the loss of lysine was attributed to destruction and half to the formation of bonds within the protein molecule which resisted attack by proteolytic enzymes The deterioration of methionine appeared to be caused by the formation of enzyme-resistant linkages within the protein molecule In the study of Hankes *et al* (*22*), casein was autoclaved for 20 hours in the absence of carbohydrates The rate of release of lysine by enzymes was markedly reduced by the heat treatment, whereas other amino acids were not so severely affected

In vitro analytical procedures for determining amino acid availability based on enzymatic release of amino acids from proteins have been deveoped by Sheffner *et al* (*64*) and Mauron *et al* (*43*)

Sheffner *et al* (*64*) studied the relationship between the pattern of amino acids released by digestive enzymes and the biological value of food proteins An amino acid index was described which attempts to take into account the physiological availability of amino acids during digestion The index combines the pattern of essential amino acids released by in vitro pepsin digestion with the amino acid pattern of the pepsin resistant residue to produce an integrated index termed the pepsin digest residue (PDR) amino acid index PDR index values were closely correlated with NPU values for a variety of proteins Division of the PDR index by the digestibility coefficient of the respective proteins yielded values which accurately predicted the biological value of the proteins studied, as determined by nitrogen balance techniques in the rat

The conditions established by Sheffner *et al* (*64*) for obtaining in vitro pepsin digests were determined from feeding experiments with rats, which indicated that approximately 30% of ingested egg protein nitrogen is absorbed before the chyme has reached the area of the intestine where tryptic activity is significant The quantity of pepsin used and the duration of incubation in the in vitro procedure therefore were adjusted to produce approximately 30% release of microbiologically available essential amino acids from egg protein With pepsin of the proper activity the conditions are such that small variations in time, temperature, and quantity of enzyme did not appear to cause significant variation in the results

Although Sheffner's procedure appears to give results which agree with those found in the rat the amount of work involved in the method renders it unacceptable for routine evaluation of available amino acids in foodstuffs

The procedure of Mauron *et al* (*43*) is concerned with measurement of only three essential amino acids—lysine, methionine, and tryptophan—the amino acids which are most likely to be limiting in foodstuffs Briefly, the method is as follows

The sample is first dialyzed against tap water to eliminate substances of low molecular weight and then digested in the dialysis bag with pepsin at 37° at pH 2 After 15 hours of pepsin digestion the sample is digested for 24 hours with pancreatin at 50° and pH 7 to 7 5 The dialyzed fractions containing the amino acids are siphoned every hour and analyzed by appropriate procedures Tryptophan and methionine are determined colorimetrically, lysine with a specific decarboxylase, and amino nitrogen gasometrically

This method has been extensively used by Mauron *et al* (*42, 43*) for determining the availability of amino acids in processed foods of vegetable and animal origin and appears to provide useful information

In Vivo Methods Although in vitro results are of value in providing information on the release of amino acids, they obviously cannot be used directly as an index of availability in the intact animal As a result, many investigators have used in vivo procedures to measure the availability of amino acids in foods Schweigert and Guthneck (*20, 62, 63*) used the growth of protein-depleted rats and fecal excretion of amino acids to determine the availability of lysine and methionine in foods Purified rations containing oxidized casein or sesame meal plus diammonium citrate and amino acids were used as basal diets deficient in methionine or lysine, respectively The weight gains of protein-depleted rats receiving the basal rations plus graded levels of pure lysine and methionine were compared with those of animals given equivalent amounts of these amino acids in foods to provide an estimate of the availability of the protein bound amino acid The reproducibility of results obtained with different levels of the test product indicated that this procedure could be used to study the utilization or availability of amino acids from foods Since Schweigert's early studies, growth procedures using rats or chicks have been used by many investigators to determine the availability of amino acids in foods Factors influencing availability values obtained from growth studies have been reviewed by Harper and de Muelenaere (*23*), and are therefore not described here in detail

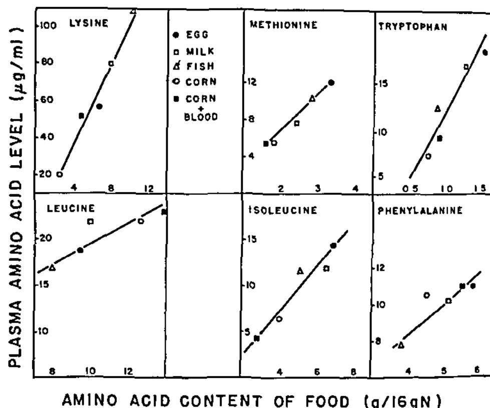
Kuiken and Lyman (*27*) estimated the availability of the ten essential amino acids in different foods by measuring the excretion of amino acids in the feces The amounts of endogenous amino acids in the feces were calculated from control periods during which the animals received a low-protein diet containing small amount of egg protein Using this technique, Kuiken (*26*) later demonstrated effects of processing on the

availability of essential amino acids in cottonseed meal Watts *et al* (*67, 68, 69*) evaluated amino acid availability in human subjects following a procedure similar to that of Kuiken and Lyman (*27*)

The interpretation of any study involving the amino acid content of feces is limited by the inability to measure the extent of degradation and synthesis of amino acids by intestinal bacteria Intestinal microorganisms may alter the amino acid distribution in food residues as they pass through the intestine Furthermore endogenous losses of amino acids by subjects receiving a low protein diet may be markedly different from those of the same subjects receiving an adequate diet The problems of relating availability to fecal excretion of amino acids are even more difficult in coprophagic animals such as the rat where fecal amino acids may be recycled through the animal

The availability of an amino acid in foods also can be determined by measuring nitrogen balance during alternate periods of feeding a basal diet supplemented with equivalent amounts of the amino acid in free or protein-bound form (*34*) Using this technique Linkswiler *et al* (*31 32, 33*) showed that valine threonine and isoleucine in corn are fully available to man The nitrogen balance method is time-consuming and expensive since large quantities of pure essential amino acids are required At the same time, it suffers from the defects usually associated with a nitrogen balance study Specifically it may be questioned whether the nitrogen balance technique is sufficiently sensitive to detect relatively small changes in availability since a number of rat growth studies indicate isoleucine to be partially unavailable in corn (*11*)

Plasma Amino Acid Methods Early studies by Wheeler and Morgan (*70*) and Guggenheim *et al* (*19*) indicated that the levels of free amino acids in the plasma after a test meal may provide information on the in vivo availability of the dietary amino acids Investigations carried out in our laboratory (*38*) on adult humans showed that peak plasma lysine and methionine levels were much higher after a meal of alcohol-extracted fish solids than after a meal of fish extracted with 1,2-dichloroethane The latter sample was known from other studies to contain unavailable amino acids (*52 54*) Smith and Scott (*65 66*) reported that chicks fed overheated fish meal had lower plasma concentrations of lysine and threonine than those receiving unheated fish meal In other studies with rats, McLaughlan (*39*) observed a high degree of correlation between plasma levels of lysine, methionine tryptophan, leucine isoleucine and phenylalanine and the amounts of these amino acids in a test meal Data which illustrate those findings are summarized in Figure 3 From the preliminary information available therefore, it appears that measurement of plasma amino acid levels may provide a very useful index of the availability of amino acids in vivo



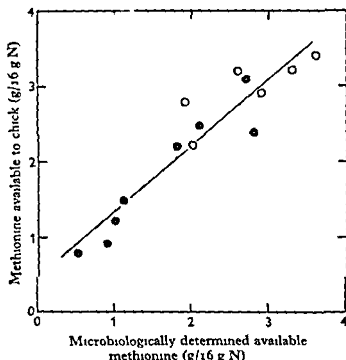
Courtesy J M McLaughlin

Figure 8 Relation between plasma amino acid level and amino acid content of foods

Microbiological Methods *Streptococcus symogenes* NCDO592, a vigorously proteolytic micro-organism, was used by Ford (*15*) to measure the biological availability of methionine, histidine, leucine, isoleucine, arginine, tryptophan, and valine in different food proteins The foods were hydrolyzed with papain before assay with the microorganism Ford (*15*) recommended use of 1 ml of 1% solution of crude papain for each 100 mg of nitrogen in the sample Values obtained for available amino acids in 12 samples of whale meal correlated closely with each other, with NPU values, and with available lysine values obtained for the same materials by Bunyan and Price (*5*)

A number of workers (*9, 57*) have noted that the extent of hydrolysis of the test protein by papain exerts a profound effect on the amount of available methionine as determined by Ford's procedure In order to obtain consistent results, therefore, it is necessary to define closely the conditions of papain predigestion and to use that amount which gives results correlating closely with available methionine values determined from studies in higher animals For example, Miller *et al* (*46*) noted that estimation of available methionine by treatment with 0 09% crude papain followed by *Strep zymogenes* assay significantly underestimated the chick

value for available methionine by 28% The values obtained afte
ment with 0 36% papain, however, were not significantly differe
those found in the chick The close correlation between the microbi
and chick values for available methionine is shown in Figure 4
from the paper of Miller *et al* (*46*) These results show that a
available methionine with *Strep zymogenes* following papain pred
can provide useful information on the availability to the growing
of methionine in highly processed animal feeds Nevertheless,
of the danger in extrapolating from results obtained with microor
to those found with higher animals, it is necessary to check microbi
values against those found in animal assays for the products in que



Courtesy British Journal of N

Figure 4 Relation between values obtained biologically (chick) and microbiologically (Streptococcus zymogenes) for available methionine in protein concentrates

Response metameter for calculating results of chick assays was food conversion e
Microbiological values obtained by assays with Strep zymogenes after predigesti
0 36% crude papain
○ *Laboratory preparation of cod muscle*
● *Commercial meat fish and whale meals*
Least squares regression line chick value = 0 49 + 0 87 (microbiological val
shown

Some Recent Problems

Effects of Solvents. Although it is well established that excessive heating during processing may reduce the availability of amino acids in foods, relatively little attention has been paid to the effects of other processing variables on the nutritional value of proteins. It is becoming increasingly apparent, however, that careful attention must also be paid to the effects of other conditions of processing on the biological availability of amino acids in foods. This is illustrated by recent studies on fish protein concentrate (FPC), a highly processed food which represents a potentially important source of high quality protein for use in underdeveloped areas of the world. Many investigators (*44, 53*) have observed marked variability in nutritional quality of the protein in samples of FPC produced by various procedures from different species of fish. Morrison and Sabry (*55*) reported that measurements of net protein ratio and PER indicated marked variability in the nutritional value of the protein in eight samples of FPC. The availability of lysine in the samples was examined by *in vitro digestion studies, animal growth assays,* and Carpenter's DNFB procedure (*6*) (Table II). One sample (Sample S) which from its color appeared to have been damaged by excessive heating during processing, contained unavailable lysine, as shown by the DNFB procedure, *reduced release of lysine during enzymic digestion of the sample,* and rat bioassay results. Another sample (Sample Z) also showed a relatively low content of available lysine as determined by the DNFB procedure. The ratio of available lysine to total lysine in this sample was not reduced, however, and rat studies using two bioassay procedures did not indicate reduction in lysine availability. These findings illustrate the obvious fact that reduced values for available lysine obtained with the DNFB procedure may not necessarily indicate a reduction in avail

Table II. Content and Availability of Lysine in Fish Flours

Sample	*Method*: *Acid hydrolysis*	*Enzyme hydrolysis*	*DNFB*	*% Availability by Bioassay*	*Gross Protein Value*
	Lysine Content, G./16 G. N				
X	8.7	5.8	7.4	88	5.99 + 0.44[a](101)[b]
Y	7.1	4.7	6.5	95	5.72 + 0.70 (97)
Z	6.7	4.6	6.0	92	4.28 + 0.29 (72)
S	7.0	2.9	5.3	61	2.85 + 0.66 (48)
T	8.8	5.0	7.1	83	5.72 + 0.48 (97)
U	8.9	5.4	6.9	85	5.97 + 0.48 (101)
V	9.1	5.5	7.1	94	5.41 + 0.34 (92)
W	8.7	5.2	7.0	100	5.97 + 0.54 (101)

[a] Standard error.
[b] Percentage of value for casein.

ability of lysine but merely a reduction in total amount of lysine present in the sample.

Morrison and Sabry (*55*) also examined the availability of the sulfur amino acids in the eight samples of FPC mentioned above. The results (Table III) showed that total methionine values varied from 2.5 grams per 16 grams of nitrogen (samples Z and S) to 3.2 grams per 16 grams of nitrogen (sample V) where as cystine levels varied from 0.6 to 1.0 gram per 16 grams of nitrogen. The amount of methionine released by enzymatic digestion of Sample S was less than that from the other samples. The procedure used involved treatment with the triple enzyme system of Wooley and Sebrell (*71*), followed by assay with a proteolytic strain of *Strep. zymogenes*. The in vitro results gave no indication that Sample X contained unavailable sulfur amino acids. Gross protein values determined on a methionine-deficient basal diet, however, indicated that Samples S and X contained unavailable sulfur amino acids. Furthermore, total methionine plus cystine values correlated closely with PER values for all samples except S and X. The in vitro procedure used, therefore gave a misleading impression as to the biological availability of the amino acids in Sample X. They indicate that microbiological procedures for available methionine may not reflect the availability of this amino acid to the animal and should be used with caution, unless a correlation with animal results has been established for the particular products being tested.

Table III. Methionine and Cystine in Fish Flours

(G./16 G. N.)

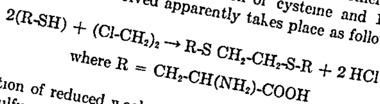
Sample	*Methionine*		*Cystine*
	Acid Hydrolysis	*Enzyme Hydrolysis*	*Acid Hydrolysis*
X	3.0	3.0	1.0
Y	2.8	2.3	0.8
Z	2.5	2.1	0.7
S	2.5	1.4	0.6
T	3.1	2.8	1.0
U	3.0	2.6	0.8
V	3.2	3.0	1.0
W	3.0	2.8	1.0

In subsequent studies (*52, 54*), it was observed that the weight gains of rats fed FPC Sample X were markedly increased by adding histidine and methionine to the diet. The amounts of methionine and histidine released during in vitro digestion with pancreatin were much less for Sample X than for a sample of FPC which was of high nutritional value (Table IV). Steaming Sample X for 30 minutes significantly increased its gross protein value determined in a methionine-deficient diet.

Table IV. Histidine and Methionine in Fish Flours X and CFF

Sample	Methionine, G./16 G. N		Histidine, G./16 G. N	
	Acid	Enzyme	Acid	Enzyme
X	3.30	1.48	1.60	0.49
CFF	3.34	2.07	2.10	1.15

Although relatively little information was available on the processing history of Sample X, it was known that the material had been prepared in a pilot plant by azeotropic extraction with 1, 2-dichloroethane. It is well established that alkyl halides can react with sulfhydryl groups of reduced wool (*17, 59*), cysteine, and glutathione (*36*), to produce thioethers. S-(Dichlorovinyl)-L-cysteine, prepared by reaction of trichloroethylene with the disodium salt of L-cysteine in liquid ammonia, produces an aplastic anemia in calves and has been implicated as the toxic factor in trichloroethylene-extracted soybean meal (*37*). Studies were conducted, therefore, to determine whether 1,2-dichloroethane or other solvents can react with the protein of fish solids during production of FPC (*54*). As shown in Table V, extraction of freeze-dried cod fillets with 1,2-dichloroethane resulted in destroying cystine and histidine and interfering with the release of cystine histidine, and methionine by pancreatic digestion. Hexane, ethanol, and isopropyl alcohol had no effects on the amino acids in fish solids. The reaction was pH—dependent, occurring most readily under slightly alkaline conditions. The thioether S,S ethylenebiscysteine was synthesized by reaction of cysteine and 1,2-dichloroethane. The reaction involved apparently takes place as follows:



$$2(R\text{-}SH) + (Cl\text{-}CH_2)_2 \rightarrow R\text{-}S\text{-}CH_2\text{-}CH_2\text{-}S\text{-}R + 2\,HCl$$

$$\text{where } R = CH_2\text{-}CH(NH_2)\text{-}COOH$$

Alkylation of reduced wool or fish solids, and subsequent hydrolysis, yielded a sulfur-containing compound with the same R_f value as the synthetic thioether. The thioether appeared devoid of biological activity for *L. mesenteroides* P-60, *Tetrahymena pyriformis* W, and the rat. It was cleaved by autoclaving but stable to acid hydrolysis.

These studies suggest that sulfhydryl groups of protein can be alkylated by 1,2-dichloroethane to produce thioether linkages, which resist hydrolysis by proteolytic enzymes. Although relatively little is known concerning the actual mechanisms by which enzymes attach to protein substrates it is possible that the reduced susceptibility to hydrolysis may be related, in part, to a reduction in the number of substrate sulfhydryl groups available as points of attachment for enzyme molecules. Furthermore if thioether bridges are established between adjacent peptide chains,

it is conceivable that the susceptibility of these peptides to proteolytic attack may be lessened.

The studies mentioned above establish clearly the important effects which solvents can exert on the availability of amino acids in foods and indicate the importance of careful evaluation of possible nutritional effects of chemicals used in processing foods.

Effects of Smoking and Salting. Dvorak and Vognarova (*13*) reported recently that the available lysine content of meat, as measured by Carpenter's DNFB procedure (*6*), was decreased markedly in products which had been smoked for 1 to 2 days. The amount of available lysine decreased proportionately with the amount of smoke used. In in vitro studies, a linear relationship was found between the amount of available lysine and the concentration of formaldehyde which is one of the constituents of wood smoke. The NPU of raw beef fillet was found to be 73, whereas that of formaldehyde treated beef fillet was only 58. Dvorak and Vognarova (*13*) concluded that the effects of formaldehyde were related to its ability to react with the free α amino groups of lysine molecules in beef protein. Similarly sodium nitrite, used for curing meat to obtain a desirable color, destroyed lysine by reaction with the free α-amino group. Since the amount of nitrite normally added to meat products is small, the reduction in available lysine content caused by nitrite addition is probably not of practical significance.

In contrast to the results obtained by Dvorak and Vognarova (*13*) Munro and Morrison (*56*) could detect no effect of salting and smoking on the biological value of cod protein as indicated by PER and gross protein values, total lysine and methionine content, available lysine values and plasma free lysine and methionine levels in human subjects given the fish products. Undoubtedly the effects of smoking on available lysine values are related to the amount of smoke to which the protein is exposed as well as to other factors.

Table V. Effects of Extracting Fish Protein for 16 Hours with Various Solvents on Methionine, Histidine, and Cystine Content

Solvent	*Methionine G/16 G N*		*Histidine G/16 G N*		*Cystine G/16 G N*	
	Acid	*Enzym*	*Acid*	*Enzyme*	*Acid*	*Enzyme*
Nil	3.6	2.33	2.67	1.24	0.97	0.46
Hexane	3.1	1.76	2.30	0.88	0.92	0.42
Isopropyl alcohol	3.3	1.88	2.32	1.15	0.92	0.40
Ethylene dichloride	3.2	0.48	1.73	0.33	0.54	0.09
Ethanol	3.4	1.96	2.38	1.15	0.90	0.39

Differential Effects on Availability of Amino Acids Under specific processing conditions, the availability of certain amino acids may be affected more than that of others Heating casein with glucose, for example, markedly reduces its nutritional value (*24, 35*), and the nutritional damage is largely corrected by supplementing with lysine (*25, 42*) It has been postulated (*30*) that the reaction between proteins and sugars involves condensation of the carbonyl groups of the sugar with free amino groups of the protein to yield indigestible linkages Considerable evidence exists that these linkages can be formed even under mild conditions (*28, 29*) Since lysine still contains a second reactive amino group even when its α-amino moiety is linked into the natural peptide chain, it is not surprising that this amino acid reacts readily with sugars

Recently, Donoso *et al* (*12*) heated pork protein with an equal weight of water, for 24 hours at 110° The material contained tritium labeled lysine, which was used to observe the fate of lysine in rats fed the heated protein Chemical analysis showed losses of dry weight, nitrogen, sulfur, and all the essential amino acids There was a 20% loss of lysine and a 25% loss of methionine plus cystine The loss of cystine (44%) was particularly severe The specific activity of the feces of rats fed the heated protein indicated reduced digestibility of lysine in the material In contrast to the results with lysine, the observed reduced utilization of the sulfur and nitrogen of the heated protein was due to reduction in the biological value thereof In other words, the unutilized portion of lysine was found in the feces, whereas the equivalent fractions of nitrogen and sulfur were found in the urine

In more recent work, Narayana Rao *et al* (*57*) utilized the casein glucose system to determine whether specific types of heat treatment preferentially affect the availability of lysine or the sulfur amino acids As shown in Table VI, they noted that autoclaving, or dry heating at 37° C or 85° C, caused marked reductions in the amount of available lysine, as determined by the DNFB method, and smaller differences in available methionine levels Narayana Rao *et al* (*57*) concluded from their studies and those of previous workers that mild heating of materials rich in reducing sugars, such as milk, causes a greater fall in available lysine than in available methionine In contrast, heating materials low in reducing sugars results in nutritional damage only if the conditions of heating are severe, and the fall in available methionine then is at least as great as that in available lysine A simple explanation for these effects would be that in the presence of reducing sugars, even mild heating results in reactions with free amino groups primarily those of lysine When reducing sugars are absent, however severe conditions are necessary to cause nutritional damage, which may result from internal ester or amide formation, or crosslinkage by hydrogen or S-S bonding More resistant bonds

whose nature is not well understood also may be formed, as suggested by Donoso *et al* (*12*) and previous workers

Table VI Available Lysine and Methionine in Casein Glucose Mixtures

Test Material	*Amino Acids G/16 G N*		
	Total methionine	*Available methionine*	*Available lysine*
No 1 (control)	4 1	3 1	5 4
No 2 (85° C for 40 min)	4 2	2 8	4 2
No 3 (37° C for 10 days)	4 1	2 7	2 4
No 4 (autoclaving at 15 psi for 30 min)	4 4	2 4	2 1

Selection of Availability Procedure It is pertinent, in view of the preceding discussion to ask which availability procedure to use in evaluating protein foodstuffs Miller *et al* (*47*) have pointed out that available methionine values for animal protein concentrates correlated closely with those for available lysine They suggested that either the chemical estimation of available lysine or the microbiological procedure with *Strep zymogenes* for available methionine will not only assess the particular amino acid involved but also give an indication of the over all value of an animal protein concentrate

However the type of food involved and the processing procedures undergone by it will determine whether or not a particular test can predict the over all protein value For example evidence cited above indicated that the solvent used in processing FPC can exert specific effects on the sulfur amino acids and that in such products measurement of available lysine may provide a misleading estimate of the nutritive value of the protein Similarly in foods which contain appreciable amounts of reducing sugars the nutritional damage caused by moderate amounts of heat during processing appears to be related primarily to binding of lysine In such products measurement of available lysine would appear to provide a more valid estimate of nutritional damage than is provided by available methionine values It is difficult to escape the conclusion that recommendations regarding the most suitable availability test must apply only to a given product processed in a specific manner

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13

Toxic Substances Associated with Seed Proteins

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The trypsin inhibitors and hemagglutinins of legumes are examples of proteins implicated in the poor nutritive value of unheated plant material A number of amino acid derivatives are the active principles responsible for various forms of lathyrism which accompany the ingestion of peas belonging to genus Lathyrus Among the glycosides, goiterogenic agents have been isolated from various cruciferous oil seeds, and cyanogenetic glycosides have been demonstrated in lima beans, linseed meal, and other legumes Glycosides in soybeans include the saponins, which have hemolytic properties, and the isoflavones, with esterogenic activity Among the miscellaneous toxic factors are gossypol (cottonseed), the causative agent of favism, the metal-binding constituent of soybean protein, and antivitamin factors in some legumes

It is ironic that while Nature has generously provided man with a liberal supply of plant protein foods, she has at the same time seen fit to contaminate these foods with a variety of substances which may be considered "toxic" to the animal body In our present discussion I do not wish to restrict myself to a definition of "toxic" that a toxicologist might use—a substance which when administered to an animal produces death at a certain level which is expressed in terms of an LD_{50} Rather I would prefer, for want of a better term, the phrase "antinutritional" to describe any adverse physiological response which is produced by the ingestion of a particular plant protein, whether it be an acute lethal effect resulting in death, or a chronic effect resulting in poor growth or some glandular disorder

It would manifestly be impossible to include a detailed coverage of all of the deleterious substances that are known to be present in plant materials I concern myself here only with plants whose seeds have present or potential

value as a source of protein for human feeding This will include such important oil bearing seeds as the soybean, peanut, cottonseed, and a number of beans and peas which form an important part of the diet in India, Africa, Central and South America, and the Mediterranean countries *Although the subject of toxic substances in legumes has been reviewed (31)*, the present paper brings the subject matter up to date and emphasizes the chemical aspects of the problem

From the point of the chemist, these toxic substances may be classified into three main categories as shown in Table I proteins or protein derivatives glycosides, and a miscellaneous group of substances of diverse but, in most cases unknown chemical structure

Table I Antinutritional Factors in Seed Proteins

I Proteins or amino acid derivatives
- 1 Trypsin inhibitors
- 2 Hemagglutinins
- 3 Osteolathyrogens
- 4 Neurolathyrogens

II Glycosides
- 5 Goitcrogens
- 6 Cyanogens
- 7 Saponins
- 8 Isoflavone glycosides

III Miscellaneous
- 9 Gossypol
- 10 Causative principle of favism
- 11 Metal binding factor
- 12 Antivitamin factors

Proteins and Protein Derivatives

Trypsin Inhibitors Perhaps the best known and certainly the most studied of all of the antinutritional factors is a trypsin inhibitor first isolated from the soybean by Kunitz in 1945 (*28*) This is a protein having a molecular weight of about 20 000, and it forms an inactive complex with trypsin Since Osborne and Mendel in 1917 (*47*) had noted that raw soybeans supported growth poorly unless subjected to heat treatment it was logical to assume that the trypsin inhibitor was the substance responsible for the poor nutritive value of the unheated bean The hypothesis that its effect could be readily explained on the basis of its ability to inhibit intestinal proteolysis was an appealing one The fact that methionine also markedly improved the nutritive value of raw soybeans (Table II) was taken to indicate that the trypsin inhibitor somehow interfered with the availability or utilization of methionine from the raw legume

Table II Effect of Heat and Methionine on Nutritive Value of Soybeans (*34*)

Diet	*Protein Efficiency*
Raw soybean meal	1 33
Autoclaved soybean meal	2 62
Raw soybean meal + 0 6% methionine	2 42

It now appears that the explanation for the growth-inhibiting property of the trypsin inhibitor is not a simple one, and, although 20 years have elapsed since Kunitz first isolated his trypsin inhibitor, there is still a decided lack of agreement as to the mechanism whereby the trypsin inhibitor exerts its effect Furthermore, there are at least four trypsin inhibitors (*52*), and it is not certain whether all four produce the same physiological effects There is little doubt that pancreatic hypertrophy is one of the primary physiological effects of feeding raw soybean (*12*) Booth *et al* (*7*) are of the opinion that pancreatic hypertrophy leads to an excessive loss of endogenous protein in the form of exocrine protein secreted by the pancreas Since this protein is rich in cystine, this represents a net loss of cystine from the body This increased need for cystine for protein biosynthesis during pancreatic hypertrophy is reflected by an increase in the conversion of methionine to cystine in the pancreas (*4*) This would explain why the need for methionine is particularly acute in diets containing raw soybeans

Trypsin inhibitors have also been found in a large number of other legumes including the peanut, navy bean, lima bean, and the various grams and pulses eaten in India, but the exact nutritional significance of these inhibitors is not clear In fact there appears to be no clear cut correlation between the trypsin inhibitor content of legumes and the beneficial effect of heat on their nutritional value (*8*)

Hemagglutinins It was this lack of correlation that led our own laboratory to search for some other factor in legumes which might account for the poor growth-promoting quality of most legumes which are not heated We succeeded in isolating from raw soybeans a protein which had the rather peculiar property of being able to cause red blood cells to agglutinate (*35*) Such substances have been known for some time to be present in many plants and are referred to as phytohemagglutinins (*32*) Some of these are highly toxic, such as ricin from the castor bean (*62*) Another fairly well known hemagglutinin is concanavalin A, which was first crystallized from the jack bean by Sumner (*61*) Landsteiner had reported many years ago that even such common edible legumes as the navy bean, lentil, and garden pea also contained these hemagglutinins (*32*)

Until we got interested in the hemagglutinins over 10 years ago, the nutritional significance of these substances was largely overlooked

In 1963 we showed that the purified soybean hemagglutinin was capable of significantly inhibiting the growth of rats (Table III) It would appear, however, from the work of Birk and Gertler (*6*) that the growth impairment of rats and chicks fed raw soybean meal may be due largely to factors

Table III Growth Inhibitory Effect of the Soybean Hemagglutinin (SBH) (*33*)

Protein Component of Diet	*Wt Gain in 2 Weeks, G*	*% Growth Inhibition*
25% heated soybean meal	60 0	0
25% raw soybean meal	28 0	43 2
25% heated soybean meal + 0 8% SBH	45 0	25 6

other than the soybean hemagglutinin These authors reported that the insoluble residue which remained after extraction of the raw meal with acid at pH 4 2 retained the capacity to inhibit growth, despite the fact that this fraction exhibited little hemagglutinating activity

Table IV compares some of the physical properties of the soybean hemagglutinin (SBH) with ricin and concanavalin A SBH has been shown to be a mucoprotein containing about 5 to 6% carbohydrate, made up largely of mannose and glucosamine (*37*, *64*) It also appears to be composed of two chains with alanine residues at each of the *N* termini and serine and alanine at the *C* termini (*64*)

Table IV Comparison of the Physical Properties of SBH with Ricin and Concanavalin A

Hemagglutinin	*Source*	*Sedimentation Constant S_{20} w*	*Diffusion Constant, D_{20} w*	*Mol Weight*	*Isoelectric Point pH*	*Ref*
SBH	Soybean	6 4	5 7	96 000	6 1	(*48*)
Ricin	Castor bean	6 4	6 0	98 000	5 5	(*62*)
Concanavalin A	Jack bean	6 0	5 6	96 000	5 5	(*61*)

When we turned our attention to the hemagglutinins from other legumes the nutritional significance of these substances became even more apparent We were particularly interested in legumes which enjoy popular consumption in underdeveloped countries For this purpose, we were able to obtain a black bean from Guatemala Bengal and red gram from India mung bean from the Philippines and the domestic kidney bean The effect of heat on the nutritive value of these beans is shown in Table V and the hemagglutinating and antitryptic activities are shown in Table VI Only the growth promoting values of the black bean and kidney bean were

Table V Effect of Heat on Nutritive Value of Some Legumes (22)

Source of Protein	*Gain in Weight, G/Day*	
	Raw	*Heated*
Phaseolus vulgaris		
Black bean	−1 94 (4–5)	+1 61
Kidney bean	−1 04 (11–13)	+1 48
Cicer arietinum		
Bengal gram	+1 25	+1 16
Cajanus cajan		
Red gram	+1 33	+1 74
Phaseolus aureus		
Mung bean	+1 05	+1 07

ᵃ 100% mortality observed during period (in days) shown in parentheses

Table VI Hemagglutinating and Antitryptic Activities of Crude Extracts of Raw Legumes (22)

Legume	*Hemagglutinating Activity HU/Ml*	*Antitryptic Activity TIU/Ml*
Phaseolus vulgaris		
Black bean	2450	2050
Kidney bean	3560	1552
Cicer arietinum	0	220
Cajanus cajan	0	418
Phaseolus aureus	0	260

ᵃ HU = hemagglutinating units, TIU = trypsin inhibitor units as defined by Honavar *et al* (22)

improved by heat, and these beans were the only ones which displayed a significant level of hemagglutinating activity For these reasons, the hemagglutinin was purified from each of these beans and fed at various levels to rats in a basal ration containing 10% casein (Table VII) A definite inhibition of growth was apparent at levels as low as 0 5% of the diet, the kidney bean hemagglutinin (KBH) being much more effective than the black bean hemagglutinin (BBH) In fact, KBH at a level of 0 5% caused 100% mortality after about 2 weeks, whereas 1 2% BBH was necessary to produce a similar rate of mortality Data are also included which show that the toxicity of the hemagglutinins may be inactivated by heat

As to the mechanism whereby the hemagglutinin exerts its effect, present evidence would suggest its site of action to be the lining of the intestinal tract Jaffé (25) noted a definite impairment in the absorption of protein and fat when rats were fed raw black beans or the hemagglutinin

purified therefrom. He was able to show that isolated intestinal loops taken from rats fed the raw bean or the purified hemagglutinin absorbed glucose at about half the rate of loops taken from control animals. These results would indicate that the hemagglutinin might combine with the mucosal cells lining the intestinal wall, thus interfering with the absorption of essential nutrients.

Table VII. Effect of Purified Hemagglutinin Fractions from the Black Bean and Kidney Bean on Growth of Rats (26)

Source of Hemagglutinin	*Purified Hemagglutinin in Diet, %*	*Av. Gain in Weight, G./Day*	*Mortality, Days*
Black bean	0	+2.51	
	0.5	+1.01	
	0.5[b]	+2.37	
	0.75	+0.20	
	1.2	−0.91	15–19
	2.3	−1.61	12–17
	4.6	−1.72	5–7
Kidney bean	0	+2.31	
	0.5	−0.60	13–16
	0.5[b]	+2.29	
	1.0	−0.87	11–13
	1.5	−1.22	4–7

100% mortality observed during period recorded. Blank space indicates no deaths observed.

[b] Solution of hemagglutinin boiled for 30 minutes and dried coagulum fed at level indicated. Hemagglutinating activity was completely destroyed by this treatment.

Amino Acid Derivatives

Lathyrism. Lathyrism is a disease associated with the consumption of certain species of peas belonging to the genus *Lathyrus*. A graphic description of this syndrome in India in the 1830's is provided by Sleeman (2, 58). In 1829 and in 1831 the wheat crop failed in Sangor, and the inhabitants subsisted mainly on *L. sativus* which grew wild in the blighted fields. By 1833 the younger part of the population of this area began to be deprived of the use of their limbs below the waist by paralytic strokes. None attacked recovered the use of their limbs.

In more recent times this disease has afflicted some segments of the population in India and the Mediterranean area and is generally associated with the consumption of *L. sativus* (chickling vetch), *L. cicera* (flat podded vetch) and *L. clymenum* (Spanish vetch). The precise causative agent of human lathyrism has not been easy to elucidate because of the difficulty with which this disease can be produced in animals. The aforementioned species of *Lathyrus* which have been implicated in human lathyrism are relatively nontoxic to most animals (28), although two other species of

Lathyrus—*L latifolius* (perennial sweet pea) and *L sylvestris* (flat pea)—produce neurological symptoms in rats which resemble those of human lathyrism (*30, 54*) Three other peas, however—*L odoratus* (sweet pea), *L pusillus* (singletary pea), and *L hirsutus* (Caley pea)—produce skeletal deformities in rats, symptoms which are different from human lathyrism (*29*)

It is evident, therefore, that the concept of what actually constitutes lathyrism is not a simple one Some clarity is achieved if one accepts the conclusion of Selye (*56*) that the consumption of *Lathyrus* can produce two distinctly different types of diseases, a "neurolathyrism" which involves damage to the central nervous system, and an "osteolathyrism" which affects bone and connective tissue This permits the classification of lathyrogenic seeds as shown in Table VIII The whole problem of

Table VIII Classification of Lathyrogenic Seeds According to Their Physiological Effects

(Neurolathyrism)

In man	In rats
L sativus (chickling vetch)	*L latifolius* (perennial sweat pea)
L cicera (flat podded vetch)	*L sylvestris* (flat pea)
L clymenum (Spanish vetch)	*V sativa* (common vetch)

(Osteolathyrism)

In rats only

L odoratus (sweet pea)
L pusillus (singletary pea)
L hirsutus (Caley pea)

lathyrism has been considerably clarified in recent years by the isolation and characterization of what appear to be the causative principles of these two forms of lathyrism

In 1954 two groups of workers, Dupuy and Lee (*16*) and McKay *et al* (*39*), isolated a compound from *L odoratus* and *L pusillus* which was capable of reproducing all of the symptoms associated with osteolathyrism This proved to be β-(*N*-γ-glutamyl)-aminopropionitrile although it was subsequently shown that β aminopropionitrile (BAPN) was the active portion of the molecule (see Figure 1) This compound was notably absent from *L sativus* and the other species of lathyrus known to cause human lathyrism

In 1961, Ressler *et al* (*53*) reported the isolation of α, γ-diaminobutyric acid (Figure 2) from *L latifolius* and *L sylvestris* which had previously been found to reproduce the neurotoxic symptoms similar to that noted in man α,γ-Diaminobutyric acid produced the same symptoms as the

seeds themselves Strangely enough, however, this compound could not be detected in species implicated in human lathyrism

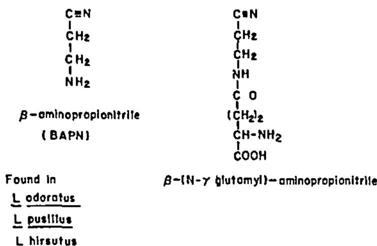


Figure 1 Structure of compounds causing osteolathyrism in rats

Ressler's group has also isolated from *Vicia sativa* (common vetch) β-cyano-L-alanine (Figure 2) which also produced neurotoxic symptoms in rats—namely tremors, convulsions, rigidity, and finally death *V sativa* has been frequently reported to be a common contaminant of *L sativus* in human outbreaks of human lathyrism in India (*3*) Ressler

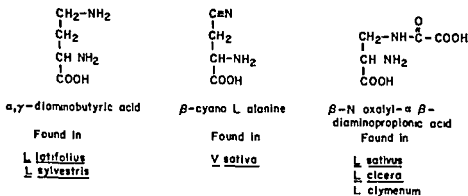


Figure 2 Structure of compounds causing neurolathyrism in rats

has therefore suggested the possibility that the causative factor in human lathyrism may be the cyanoalanine provided by the common vetch as a contaminant of *L sativus rather than by L sativus itself*

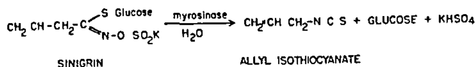
Two groups of workers in India (*1, 48*) have recently reported the isolation of a neurotoxic principle from *L sativus* itself which was identified as β-*N*-oxalyl-α,β-diaminopropionic acid (Figure 2) Intraperitoneal injections of this compound produced neurotoxic symptoms in chicks manifested by an inability to stand, head retraction, paralysis of the legs, and convulsions α,β-Diaminopropionic acid itself proved nontoxic, showing the essentiality of the oxalo group for toxicity A final judgment as to whether this compound is the long-sought-for causative principle *of human lathyrism must await further studies*

Glycosides

Goiterogenic Agents Sometimes the adverse physiological effects of certain legumes do not manifest themselves in such obvious ways as growth inhibition, paralysis, or death A case in point is the observation that unheated soybeans produce a marked enlargement of the thyroid gland of the rat and chick, an effect which can be counteracted by the *administration of iodide or partially eliminated by heat (49, 57)* Several workers (*23, 63*) have reported a number of cases of goiter in human infants fed soybean milk Apparently the heat treatment employed for sterilizing these particular soybean preparations was not sufficient to destroy the goiterogenic agent Iodine supplementation, however, alleviated this goiter condition in human infants (*63*) No goiterogenic agent has ever been isolated from soybeans, so it is not known what substance is actually responsible for the goiterogenicity of raw soybean

On the other hand, the goiterogenic properties of peanuts have been attributed to a phenolic glycoside which is present in high concentrations in the red skin (60) It has been suggested that the phenolic metabolities formed from this glycoside are preferentially iodinated and thereby deprive the thyroid of available iodine

It has been known for some time that some cruciferous oilseed proteins such as rapeseed, mustard seed, and crambe (Abyssinian kale) contain thioglycosides which, upon enzymatic hydrolysis, release goiterogenic isothiocyanates As an example, the thioglycoside of the mustard seed, sinigrin, is hydrolyzed by the enzyme "myrosinase" to give allyl isothiocyanate (Figure 3) Other crucifers give related unsaturated isothiocyanates as well as cyclic oxazolidinethione derivatives which are also goiterogenic Since the release of these goiterogenic substances is mediated by enzymes already present in the meal, the meal can be moistened to permit the enzymatic liberation of the isothiocyanates, which may then



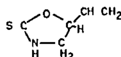


Figure 8 Structure of sinigrin and its enzymatic products according to Ettlinger and Lundeen (17)

Also shown S-5 vinyl oxazolidine 2-thione another goiterogenic product of certain thioglycosides present in some cruciferous plants

be removed by steam distillation (*44*) Unlike the goiterogenic effect of soybeans, dietary supplementation with iodide is not effective

Cyanogenetic Agents It has been known for a long time that a number of plants are potentially toxic because they contain glycosides from which HCN is released upon hydrolysis Those most commonly eaten by man or animals are listed in Table IX in order of their HCN content Acute cassava poisoning is not uncommon in the West Indies, South America and West Africa, where the consumption of this plant is high Many deaths of domestic animals have been attributed to the high HCN content of sorghum and linseed meal As far as seed proteins for human consumption are concerned the lima bean constitutes the most serious problem although lesser amounts of HCN are produced by other legumes Serious outbreaks of poisoning attributed to the consumption

Table IX Cyanide Content of Certain Plants

Plant	*HCN Yield Mg/100 G*
Lima bean (*Phaseolus lunatus*)	
Samples incriminated in fatal human poisoning	210 -312
Normal levels	14 4-16 7
Sorghum	250
Cassava	113
Linseed meal	53
Black-eyed pea (*Vigna sinensis*)	2 1
Garden pea (*Pisum sativum*)	2 3
Kidney bean (*Phaseolus vulgaris*)	2 0
Bengal gram (*Cicer arietinum*)	0 8
Red gram (*Cajanus cajans*)	0 5

of lima beans have been reported from time to time in India, Puerto Rico, Mauritius, and even Europe (*42*)

The HCN is derived from cyanide-containing glycosides which upon hydrolysis yield sugar, HCN, and either acetone or benzaldehyde As shown in Figure 4, phaseolunatin, the cyanogenetic glycoside of the lima bean, yields HCN, glucose, and acetone when hydrolyzed by the enzymes β-glucosidase and oxynitrilase (*15, 50*) These enzymes are contained in the plant, so that lima beans should be detoxicated by adequate cooking Yet many cases of human intoxication have occurred even with cooked lima beans It has been reported (*19*) that when lima beans, which had been cooked so as to destroy the enzymes responsible for cyanide formation, were fed to human subjects, cyanide could be detected in the urine This has led to the supposition that perhaps enzymes secreted in the intestinal tract or by the microflora of the colon may be responsible for releasing HCN after ingestion of the beans

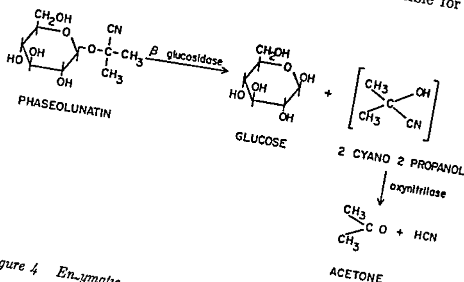


Figure 4 Enzymatic products of phaseolunatin the cyanogenetic glycoside of the lima bean

Saponins Saponins are bitter-tasting, foam-producing glycosides in which the nonsugar residue (sapogenin) is a triterpenoid alcohol referred to as a soyasapogenol Present evidence would indicate that at least five different saponins (*65*) exhibit varying degrees of hemolytic and foam producing activity (*20*) The structures of the five soyasapogenols derived from these saponins are shown in Figure 5

Because of their characteristic hemolytic activity and bitter taste, the soybean saponins have been thought to be one of the factors contributing to the poor nutritive value of unheated soybean protein (*61*) Birk *et al* (*5*), however, have shown that the hemolytic activity of the soybean saponins is unaffected by the heat treatment necessary to produce optimum nutritive value of soybean meal This would indicate that the hemolytic

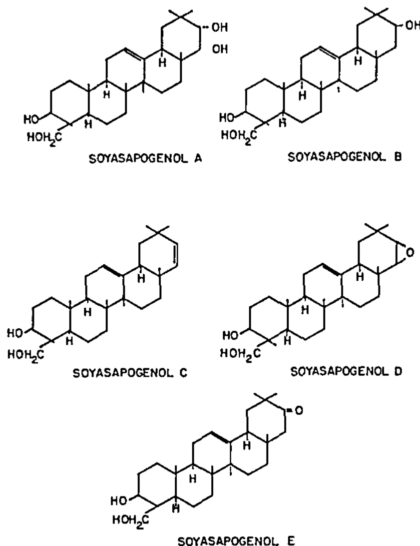


Figure 5 Structures of soyasapogenols A, B, C, D and E

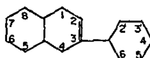
Structures for soyasapogenols A B C and D taken from Smith et al (59) structure for soyasapogenol E taken from Willner et al (65)

property of the saponins is of little importance in determining the nutritive value of soybean meal High levels of soybean saponin also inhibit the proteolytic activities of trypsin and chymotrypsin (24) This inhibition however, is believed to be the result of a nonspecific reaction of the saponins with protein, since preincubation of a soybean saponin extract with various protein substrates prior to the addition of the enzyme could abolish this inhibitory effect Thus the soybean protein which normally accompanies the saponin in the meal would be expected to negate any detrimental effect of saponins on digestive processes in vivo

Four sapogenins have also been isolated from the saponins of the peanut three appear to be different from the soyasapogenols, whereas one

is identical in chromatographic behavior to soyasapogenol B (*14*) Whether these peanut saponins are of any physiological significance in the consumption of peanuts remains to be demonstrated

Isoflavone Glycosides A number of isoflavones occur in soybeans in the form of their glycosides (*13*) The structures of those thus far characterized are



ISOFLAVONE

Genistein	4,5 7 trihydroxyisoflavone
Daidzein	4 7 dihydroxyisoflavone
Isogenistein	2 5 7 trihydroxyisoflavone
Tatoin	8 methyl 4 5 dihydroxyisoflavone
Methylgenistein	8 methyl 4 5 7-trihydoxyisoflavone
Methylisogenisten	8-methyl 2' 5 7-trihydroxyisoflavone

The sugar moiety is attached at one or more of the positions occupied by the hydroxyl groups in the free isoflavone Genistein and the glucoside of genistein, genestin, have been demonstrated to have estrogenic activity when tested on female rats and mice (*10*, *45*) Commercial soybean oil meal itself is estrogenically active, but the meal which has been extracted with methanol to remove genistin is inactive (*11*) In addition to their estrogenic activity, both genistin and genistein isolated from soybean meal and fed at a level of 0 5% of the diet produce in the male rate (*41*) a decrease in weight gain of the entire body as well as the kidney and spleen, an increase in the iron content of the liver and spleen, elevated levels of zinc in the liver and bones, and increased deposition of calcium, phosphorus, and manganese in the bones

Other Anti-Nutritional Factors

Gossypol In contrast to the widespread distribution of the trypsin inhibitors and hemagglutinins among the legumes, cottonseed contain the toxic substance, gossypol, which is peculiar to the pigment glands of the cottonseed

The toxicity of cottonseed meal is more or less proportional to its gossypol content For this reason, cottonseed meal was for many years considered an undesirable protein supplement for monogastric farm animals As a result of intensive research, suitable processing techniques

pound is responsible for a disease which to our knowledge can be produced only by the ingestion of this particular bean

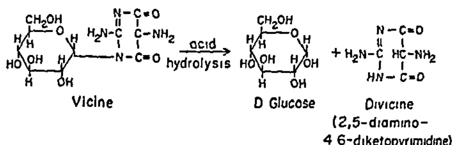


Figure 6 Structure of vicine and its hydrolytic products

Metal-Binding Constituents Preparations of isolated soybean protein have been shown to interfere with the availability of certain mineral constituents of the diet—zinc, manganese, copper, and iron (*18*, *46*) The exact mechanism whereby soybean protein exerts this effect is un known, although it may be related to the observation that a soybean protein-phytic acid complex has a special affinity for metal ions (*46*)

Antivitamin Factors Carlson *et al* (*9*) have recently reported that unheated soybean protein, or the protein isolated therefrom, caused rickets in turkey poults as judged by a decrease in bone ash This rachitogenic effect could be overcome by supplementation with vitamin D_3 or by autoclaving the meal Calcium and phosphorus were ineffective in this respect

According to Hintz and Hogue (*21*) raw kidney beans at a level of 50% of the diet increased the vitamin E requirements of chicks, as evidenced by the incidence of muscular dystrophy The antivitamin E effect could be partially eliminated by autoclaving the beans for 60 minutes at 127° By means of alcohol extraction two factors with antivitamin E activity could be demonstrated, one which was alcohol-soluble and relatively heat-stable and another which was heat-labile and insoluble in alcohol

Linseed meal has been shown to depress the growth of chicks, an effect which can be prevented by extracting the meal with water, autoclaving for 30 minutes at 15 pounds pressure, or supplementing with pyridoxine (*26*, *27*) *The identity of this antipyridoxine factor is yet* to be determined

Conclusions

There has been much concern in recent years with the possible toxic effects of chemicals added to foods, with relatively little attention being paid to toxic substances that may be present as natural components of

food substances We are in a way fortunate that these toxic substances have been added by the good Lord and not by man, otherwise we might have the food faddist to contend with in our efforts to expand the use of plant proteins Only after the presence of these toxic factors has been recognized and studied can effective measures be taken so that the full nutritional potential of the seed proteins can be realized And therein lies a good measure of the hope that we will be able to combat the threat of protein malnutrition that faces the world of tomorrow

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14

Mycotoxin Contamination of Foodstuffs

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Toxic metabolites (mycotoxins) of spoilage fungi growing on food commodities have caused acute and chronic toxicity syndromes in animals and man. "Alimentary toxic aleukia," a mycotoxicosis of man, results from ingestion of metabolites of fungi growing on cereal grains. Fungal spoilage of rice has resulted in luteoskyrin and a chlorine-containing peptide, toxic metabolites of Penicillium islandicum. The syndrome "facial eczema" of ruminants is caused by sporidesmin, a hepatotoxic metabolite of Pithomyces chartarum which grows on rye grass pastures. The aflatoxins, metabolites of A. flavus, are potent hepatotoxins in many animal species and also hepatocarcinogens in the rat, duck, and rainbow trout. Mycotoxin contamination can present real or potential health hazards and therefore impair maximum utilization of available protein sources and other foodstuffs.

Among the factors which must be established in the utilization of protein sources for human feeding is the absence of chemical substances which might have deleterious effects upon the health of the consumer. Such substances may derive from several sources, including the presence of normal genetically determined components of plants and animals used as foods. The toxic substances associated with seed proteins discussed elsewhere in this symposium are excellent examples of this type of toxic component. Also important among this group are such familiar compounds as gossypol, cycasin, tetrodotoxin, and the *Senecio* alkaloids. These substances have sometimes presented serious difficulties in the utilization of potentially significant protein sources. However, their occurrence is predictable and, in many instances, technological advances have made it possible to circumvent problems posed by their presence.

Mycotoxins are among a different group of substances important in this connection—namely, the so-called inadvertent or unintentional

contaminants which occasionally find their way into the food chain. This type of contamination poses more difficult problems in safety evaluation. The frequency and level of contamination are sporadic and the compounds as well as their toxic manifestations are frequently ill defined and diverse in nature.

The mycotoxins comprise a group of toxic secondary metabolites elaborated during the growth of certain contaminating fungi on foods or foodstuffs.

The majority of food raw materials are liable in some degree to growth of contaminating fungi at some stage during their harvest, storage, transportation, or processing. Since mold spores are ubiquitously distributed, the possibility for sporulation and subsequent growth of the fungal flora on a given product is determined principally by moisture content of the substrate, relative humidity, and temperature. During the entire postharvest period, food crops are essentially in a state of storage, and mold damage is avoidable only by careful regulation of moisture content, temperature and other environmental conditions. Commodities particularly prone to damage include grains, oilseeds, fruits, and vegetables. One recent estimate (26) reported the annual worldwide grain loss from all causes to be in the order of 55 million metric tons. Fungal spoilage contributed heavily to these losses. The annual postharvest loss of fruits and vegetables in the United States has been estimated (76) to be equivalent to the output of 13% of the producing acreage. In this instance also, mold damage constitutes a major factor in the loss.

Many types of crop damage result from mold growth, which diminishes or destroys the usefulness of the commodity as a food. For example, serious losses of tropical foods attributed to mold damage include the discoloration of rice and cocoa butter, loss of seed viability, mustiness of coffee beans, and lipolysis of plant oils (56).

While these deleterious effects have had serious economic consequences, fungal spoilage has in several instances, created additional problems by contamination of the affected commodity with mycotoxins. Toxicity syndromes, recently given the generic name "mycotoxicoses" (28), resulting from the ingestion of such contaminated foodstuffs have been encountered in many forms. All of these have either posed direct hazards to human consumers or have caused significant losses in domestic animals fed contaminated feeds. The net result in either case has been a reduction in the utilizable food supply by directly limiting human consumption or by decreasing available animal protein.

It would not be possible, nor particularly germane to this discussion, to attempt a complete review of all of the recognized mycotoxins and mycotoxicoses. These have recently been reviewed in detail (28, 46, 84, 85). Several examples have therefore been selected to illustrate the range of variation in form and character encountered in this type of syn-

drome Such factors as the causative fungi, chemical nature of the toxic compounds commodities affected, and principal features of the toxicity in susceptible species will be considered

Ergotism

From a historical viewpoint, no discussion of this subject would be complete without at least a brief allusion to ergotism, the earliest recognized form of mycotoxicosis Arising from the growth of *Claviceps purpurea* on rye and other grains, epidemics of the chronic form of the toxicity were apparently frequent during the Middle Ages The first association of ergot with the syndrome was in the mid-sixteenth century It was not, however, until the 1930 s that the group of six lysergic acid derivatives (*64*) responsible for the biological effects were isolated and identified The last major incidence of ergotism in the United States was in 1825, but serious outbreaks have occurred as recently as 1926–27 in Russia and 1928 in England (*11*) Although the hazard to the human food supply has been essentially eliminated contamination of some types of pasture grasses by the mold still causes frequent problems in veterinary practice (*80*)

Alimentary Toxic Aleukia

The most recently documented mycotoxicosis in humans is the syndrome known as 'alimentary toxic aleukia" or ATA The disease has been recorded sporadically in Russia, probably beginning in the nineteenth century but occurred with special severity from 1942 to 1947 In the peak year 1944, more than 10% of the population of one district was affected, and many fatalities occurred in nine of the 50 counties of this district (*41*) Symptoms of the disease which have been described in detail (*28*), may be summarized as follows initially, local inflammation appears in the mouth pharynx and esophageal mucosal tissues, apparently the result of direct contact with the toxic substances Systemic effects appearing after 5 to 9 days include anemia and leukopenia, which grow progressively worse over a period of 3 to 4 weeks These symptoms develop sequentially with continued intake of certain lots of grains or their products If allowed to continue the toxicity terminates in severe anemia and total exhaustion of the bone marrow, with panleukopenia and agranulocytosis In many instances death results from secondary infections

This disease was found to be associated with the ingestion of grains that had overwintered in the field Millet was an extensive vector of ATA because it is widely grown in the areas affected but wheat and barley have also been implicated in a few outbreaks The causes of the

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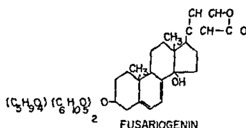
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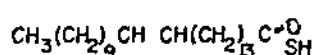
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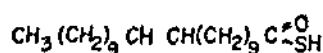
syndrome were identified as toxic compounds produced by fungi isolated from toxic grain samples (38, 39) It was originally thought that *Fusarium sporotrichioides* was the only fungus involved, but recent studies by Joffe (40) have also implicated *F poae* and *Cladosporium epiphyllum* Administration of cultures or culture extracts of these molds to various animals has revealed that the cow, sheep, and chicken are able to tolerate large amounts of toxic grain or extracts On the other hand, it has been possible to reproduce the typical human symptoms in the cat, dog, monkey, and guinea pig (28)

Numerous investigations have been concerned with the chemical properties of the toxic compounds Observations on contaminated grain samples revealed that the toxic substances are relatively stable to heat, withstanding a temperature of 118 C for 18 hours or longer Furthermore, grain samples stored for 6 years retained their toxicity The toxic compounds are extractable with lipid solvents, and a standardized biological assay procedure was developed based on rabbit dermal toxicity of diethyl ether extracts of suspected material (28) Recently, toxins produced by species of *Fusarium* and *Cladosporium* have been isolated and structural formulas proposed for them (40)



FUSARIOGENIN





EPICLADOSPORIC ACID FAGICLADOSPORIC ACID

The toxins produced by *C epiphyllum* and *C fagi* have been named epicladosporic acid and fagicladosporic acid, respectively Similarly, the toxins produced by *F sporotrichioides* and *F poae* are distinguished as sporofusariogenin and poaefusariogenin

Yellowed Rice Toxicity

Fungal damage of rice represents another significant example of the contamination of an important foodstuff by mycotoxins This crop, in common with others, is prone to mold spoilage if storage conditions permit growth of contaminating fungi The magnitude this problem can assume is illustrated by losses incurred in one major consumer country,

Japan. According to one author (78) more than 100,000 tons of rice imported during the period 1947 to 1954 were declared unfit for human consumption because of fungal damage. A systematic survey (44) of the mold flora of stored rice in Japan revealed the presence of some 200 genera and species with more than 50 penicillia represented.

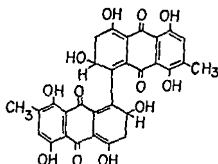
Some lots of moldy rice were found to be highly toxic to domestic and experimental animals and toxic mold metabolites were frequently identified as the causative agents. Although many of the rice fungi have been shown to produce toxic metabolites (44), three species of penicillia have been extensively studied. Ethanolic extracts of rice cultures of *Penicillium toxicarium* Miyake, when administered orally to higher vertebrates induced ascending paralysis and respiratory and circulatory disturbances resembling the symptoms of beriberi (58). Cultures of *P. citrinum* induced severe damage in the renal tubules when fed to rats, an effect attributable to citrinin produced by the mold.

A third fungus, *Penicillium islandicum* Sopp, first isolated in 1948 (58) produced severe liver cirrhosis when incorporated into rat diets. This finding was of considerable interest in view of the high incidence of liver cirrhosis and primary liver carcinoma among the Japanese and other Asiatic peoples (46) and as a consequence stimulated extensive investigation into the fungal metabolites responsible for the toxicity. Although consumption of moldy rice has not been directly associated with human disease, much useful information has resulted from these investigations.

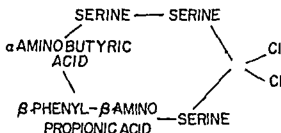
Long term studies (58) on mice fed either rice cultures of *P. islandicum* or fungal mats cultured on Czapek solution showed the presence of liver lesions ranging from acute atrophy to fully developed cirrhosis in correlation with the amount of mold culture ingested. Fully developed cirrhosis was found mostly in animals dying 220 days or later after feeding was begun. Parallel experiments in rats resulted in similar findings.

In a subsequent experiment (45) to determine whether this fungal culture was capable of hepatoma induction, moldy rice was fed to rats alone and in combination with a known hepatocarcinogen, *p*-dimethylaminoazobenzene (DAB). In animals fed only the mold culture, 5 out of 30 developed liver tumors; when fed in combination with 0.06% DAB, 14 out of 30 showed hepatomas as compared with 6 out of 30 receiving DAB alone. These results reveal the carcinogenic properties of *P. islandicum* cultures as well as their promoting effect when fed in combination with DAB.

Attempts to identify the substances responsible for these effects have resulted in isolating two toxic compounds, luteoskyrin and a chlorine-containing peptide from mycelia of the fungus (77). The structures of these compounds are



LUTEOSKYRIN



CHLORINE-CONTAINING PEPTIDE

Some of their biologic effects are summarized in Tables I and II While these compounds are clearly associated with the hepatotoxic properties of the fungal cultures, further definitive experiments are required to establish whether they are the active carcinogenic agents

Facial Eczema in Ruminants

Numerous instances of animal diseases of obscure etiology are encountered sporadically by veterinary practitioners The outbreaks usually tend to be localized and the problems are frequently solved by empirical means, and the causative factors are not elucidated However, on occasion the diseases reach epidemic proportions and consequently stimulate efforts to determine the etiologic agents involved Several such syndromes which have resulted in serious losses of animals important to the food supply have been clearly attributed to contamination of the feed by mycotoxins

Important among these is the disease known as "facial eczema," a photosensitization syndrome occurring in ruminants While the major outbreaks have apparently been restricted mainly to New Zealand and Australia, syndromes of similar, but as yet unproved, etiologic character have been encountered in the southern United States (*31*, *32*, *43*) In

Table I Toxicity of Luteoskyrin to Mice and Cell Cultures[a]

LD_{50} in Mice

Intravenous	6.65 mg./kg.
Subcutaneous	147.0 mg./kg.
Oral	221.0 mg./kg.

Liver Injury

Acute lesion centrolobular necrosis and fatty change
Chronic lesion periportal fibrosis (slight) and hepatoma formation

Tissue Culture (Chang Liver Cells)

MED[b] (growth inhibition)	0.1 μg./ml.
LD (cell destruction)	1.0 μg./ml.

[a] Adapted from Miyake and Saito (*53*)
[b] MED Minimum effective dose
LD Lethal dose

Table II Toxicity of Chlorine Containing Peptide to Mice and Cell Cultures

LD_{50} in Mice

Intravenous	338 μg./kg.
Subcutaneous	475 μg./kg.
Oral	6550 μg./kg.

Liver Injury

Acute lesion vacuolar degeneration cell destruction periportal hemorrhage
Chronic lesion liver cirrhosis monolobular

Tissue Culture (Chang Liver Cells)

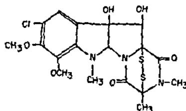
MED[b] (growth inhibition)	$10^{0.5}$ μg./ml.
LD (cell destruction)	$10^{1.5}$ μg./ml.

[a] Adapted from Miyake and Saito (*53*)
[b] MED Minimum effective dose
LD Lethal dose

New Zealand facial eczema in sheep has been a long standing problem, to which special attention was drawn in 1936, when a severe epidemic took a heavy toll of animals (*27*). The disease manifests itself clinically as a marked exudative epidermitis of the face and other exposed skin. These symptoms are sequelae of severe liver damage resulting in accumulation of phylloerythrin, a metabolic product of chlorophyll, which results in photosensitization of exposed skin areas (*54*).

Continuing efforts to establish the causative agent resulted in association of the disease with growth of the mold *Pithomyces chartarum*

on rye grass pastures used for grazing (60) In 1959, Synge and White (54) were able to isolate from this fungus a metabolite which they named sporidesmin The structure of sporidesmin has recently been elucidated (29)



This substance, when fed to guinea pigs, produced liver lesions closely resembling those caused by feeding toxic rye grass Subsequent experiments (54) have shown that the natural syndrome can also be reproduced in sheep by administration of sporidesmin The principal biochemical, functional, and histological effects of oral administration of the compound to sheep are attributable to severe liver damage Doses of 3 mg per kg of body weight are acutely toxic and most animals die within a few days Lower levels produce subacute liver damage which eventually results in photosensitization in many treated animals

The effects of sporidesmin toxicity on liver lipids in sheep have been extensively studied by Peters and Smith (61) These investigators found that a single oral dose of 1 mg per kg of body weight caused a marked increase in liver triglycerides without affecting concentrations of other lipid fractions They concluded that the early hepatotoxic action of sporidesmin was an impairment of hepatic triglyceride secreting mechanisms Studies of sporidesmin poisoning in rats (73) revealed this species to be resistant to the hepatotoxic effects and to respond in a qualitatively different manner Single doses of 6 mg per kg of body weight produced ascites and pleural effusion thought to be due to a direct effect of the toxin on capillary permeability

It is difficult to estimate the magnitude of losses of domestic animals attributable to sporidesmin or similar mycotoxins However, diseases of animals involving photosensitization are known to occur in most major livestock-producing areas of the world, and at certain times in a given area, such as New Zealand, they may be of great economic importance

Moldy Corn Poisoning

Other mycotoxicoses affecting livestock have been reported although in most cases neither the syndromes nor the etiologic relationships have been as clearly defined as in facial eczema disease One of these, the so called "moldy corn toxicosis" has been encountered sporadically in southeastern United States Outbreaks of the disease in swine were recognized as early as 1949, were particularly severe in 1952 and 1955

and have also been observed less frequently in cattle (28) In the severe outbreaks mortality varied from 5 to 55% of herds consuming toxic grain Animals dying of the acute toxicity showed signs of acute toxic hepatitis and profound hemorrhage

Burnside *et al* (16) isolated from toxic field corn two fungi which, when cultured on corn and fed to swine, produced a similar toxicity One of these was an *Aspergillus flavus* Link, and the other a strain of *Penicillium rubrum* Stoll Subsequent investigations on the toxicity of cultures of these organisms in swine revealed that the *P rubrum* strain was the more potent

The toxic substances responsible for these outbreaks have not been identified It would seem possible that the toxicity of the *A flavus* strain might be explained if this organism produced aflatoxins although there is no direct evidence leading to this conclusion In further studies on the *P rubrum* strain Wilson and Wilson (83) were able to extract and concentrate substances lethal to mice guinea pigs, rabbits, and dogs We have also been investigating cultures of this mold and have recently succeeded in isolating a crystalline compound highly toxic to mice rats, cats dogs and swine Attempts are in progress to characterize the compound and the toxicity syndrome Details of this work will be reported in full elsewhere

Aflatoxicoses

The recent discovery of the aflatoxins and their potent biologic properties has focused increasing attention on the general problem of the mycotoxins and their potential significance as food contaminants Detailed accounts of the development of knowledge of this mycotoxicosis have appeared (2 46 84) and only the major aspects of the problem are described here

Toxicity syndromes of domestic animals ultimately attributed to aflatoxins were first recognized in England in 1960 and 1961 The first report of the syndrome in young turkeys (14) was followed within a short time by similar incidents in ducklings and chickens (10) as well as swine (34, 51) and calves (52) The common factor in these episodes was a shipment of Brazilian peanut meal used in the animal rations It was found that the active principle could be extracted from toxic meals (3 67) and such extracts reproduced the toxicity in susceptible species It has since been suggested by examination of many types of agricultural commodities that peanut meals are probably more frequently contaminated than other crops (36), but the discovery of a highly contaminated cottonseed meal (50) illustrates the lack of a specific substrate-toxin production relationship

Sargeant *et al* (*66, 68*) discovered that the toxic compounds were produced by certain strains of *Aspergillus flavus* isolated from toxic meals. The generic name "aflatoxins" was subsequently applied to the group of toxic compounds produced by this fungus.

Isolation of the aflatoxins from toxic meals was greatly facilitated by the discovery that they were strongly fluorescent in ultraviolet light. This property has also provided a convenient means for monitoring isolation and purification procedures. The original investigations (*3, 67*) demonstrated that the compounds were extractable with methanol. A variety of extraction procedures have since been developed for use with various natural products or mold cultures on natural substrates, particularly in connection with chemical assays of agricultural commodities for aflatoxin contamination.

In producing and isolating quantities of aflatoxins from mold cultures on solid substrates, a convenient extraction and concentration procedure involves total extraction of the culture with chloroform and subsequent precipitation of the aflatoxins in petroleum ether (*6, 7*). The aflatoxins produced in cultures on liquid media are almost quantitatively removed by partitioning into chloroform (*1*).

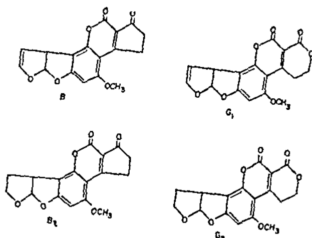
Extracts produced by these procedures usually contain mixtures of fluorescent compounds, which are separable into their individual components by chromatography. Although several systems have been developed, the conditions most widely used involve separation on silica gel plates developed with 3 to 5% methanol in chloroform (*7, 24, 56*).

When such chromatograms of extracts containing aflatoxins are viewed under ultraviolet light, an array of fluorescent compounds is generally present. The known aflatoxins comprise four of the components. Two emit blue visible light, and were therefore named aflatoxins B_1 and B_2, and two fluoresce yellow-green (aflatoxins G_1 and G_2). On silica gel plates developed in 97 to 3 chloroform methanol (*7*) aflatoxin B_1 migrates with an R_f in the order of 0.56, B_2 0.53, G_1, 0.48, and G_2 0.46, although absolute R_f values are poorly reproducible. The amounts and relative proportions of these four compounds present in culture extracts are variable depending on such factors as mold strain, medium composition, and culture conditions. Typically, aflatoxin B_1 is present in largest relative amount, with G_1 at an intermediate concentration; B_2 and G_2 usually represent less than 2% of the total yield.

These four compounds were originally isolated by groups of investigators in England (*55, 68*) and Holland (*80*). The molecular formula of aflatoxin B_1 was established as $C_{17}H_{12}O_6$ and of aflatoxin G_1 as $C_{17}H_{12}O_7$, while aflatoxins B_2 and G_2 were found to be the dihydro derivatives of the parent compounds, $C_{17}H_{14}O_6$ and $C_{17}H_{14}O_7$ (*35*).

Structures based largely on interpretation of spectral data were proposed for aflatoxins B_1 and G_1 in 1963 (*6, 7*) and for B_2 (*21, 81*) and

G_2 shortly thereafter. The proposed structure of G_1 has been supported by x-ray crystallography (*22*).



These closely related compounds are highly substituted coumarins, and the presence of the furocoumarin configuration places them among a large group of naturally occurring compounds. The bifuran structure, however, has previously been encountered in only one other compound of natural origin, sterigmatocystin, a metabolite of *Aspergillus versicolor* (*16*).

The spectral characteristics of the aflatoxins have been determined by several investigators (*6, 7, 23, 35, 80, 81*) and are summarized in Table III. The ultraviolet absorption spectra are very similar, each showing maxima at 223, 265, and 363 mμ. The molar extinction coefficients at the latter two peaks, however, demonstrate that B_1 and G_2 absorb more intensely than G_1 and B_2 at these two wavelengths. Because of the close similarities in structural configuration, the infrared absorption spectra of the four compounds are also very similar as illustrated. The fluorescence emission maximum for B_1 and B_2 has been reported to be 425 mμ and that for G_1 and G_2 is 450 mμ (*35*). The intensity of light emission varies greatly among solutions of the four compounds.

The chemical properties and reactivity of the aflatoxins have received relatively little systematic study beyond work associated with structure

Table III. Summary of Spectral Properties of Aflaxtoxins

Aflatoxin	*UV Absorption ε* 265 mμ	363 mμ	*IR Absorption (Cm⁻¹)* $\nu^{CHCl_3}_{max}$				*Fluorescence Emission* mμ
B_1	13 400	21 800	1760	1684	1632	1598	425
B_2	9 200	14 700	1760	1685	1625	1600	425
G_1	10 000	16 100	1760	1695	1630	1595	450
G_2	11 200	19 300	1760	1694	1627	1597	450

elucidation However, it has been shown (*6, 7, 81*) that catalytic hydrogenation of aflatoxin B_1 to completion results in the uptake of 3 moles of hydrogen with the production of the tetrahydrodeoxy derivative Interruption of the hydrogenation procedure after the uptake of 1 mole of hydrogen results in the production of aflatoxin B_2 in quantitative yield (*21, 81*)

Aflatoxin B_1 has also been reported to react additively with a hydroxyl group under the catalytic influence of a strong acid (*4*) Treatment with formic acid–thionyl chloride, acetic acid–ethionyl chloride, or trifluoroacetic acid results in addition products of greatly altered chromatographic properties but relatively unchanged fluorescence characteristics The presence of the lactone ring makes the compound labile to alkaline hydrolysis, and partial recyclization after acidification of the hydrolysis product has been reported (*23*)

Although few systematic studies have been carried out on the stability of the aflatoxins, the general experience would seem to indicate that some degradation takes place under several types of conditions The compounds appear partially to decompose, for example, upon standing in methanolic solution, and this process is greatly accelerated in the presence of light and/or heat Substantial degradation also occurs on chromatograms exposed to air and ultraviolet or visible light These processes may give rise to some of the nonaflatoxin fluorescent compounds typically seen in chromatograms of culture extracts The nature of the decomposition products is still unknown, and the chemical reactions involved in their formation remain to be established

The discovery of this group of compounds as contaminants of animal feeds and potential protein sources for human feeding has stimulated considerable research effort concerned with their effects in various biologic assay systems The toxic properties of the aflatoxins manifest themselves differently, depending on the test system, dose and duration of exposure Thus, they have been shown to be lethal to animals and animal cells in culture when administered acutely in sufficiently large doses and to cause histologic changes in animals when smaller doses were administered subacutely Chronic exposure for extended periods has resulted in tumor induction in several animal species

The aflatoxins are acutely toxic to most animal species Aflatoxin B_1 has been most extensively studied, as regards its lethal potency and its in vivo lethality to selected experimental animals is summarized in Table IV Early experimental studies as well as field observations suggested that the duckling was the species most susceptible to acute poisoning The LD_{50} of one-day-old ducklings is in the order of 0 5 mg per kg This value is considerably smaller than those for the rat and hamster, which are commonly used in toxicologic evaluations However, studies in our laboratories indicate that the dog, rabbit, and guinea pig have LD_{50}

Table IV. Lethality of Aflatoxin B_1 to Several Animal Species

Animal	Age	Sex	Route	LD_{50} Mg/Kg	Ref
Duckling	1 day	M	PO[a]	0.37	(20)
Rat	1 day	M F	PO	1.0	(86)
	21 days	M	PO	5.5	(86)
	100 g	M	PO	7.2	(18)
	150 g	F	PO	17.9	(18)
Hamster	30 days	M	PO	10.2	
Guinea pig	Adult	M	IP[b]	Ca. 1	
Rabbit	Weanling	M F	IP	Ca. 0.5	
Dog	Adult	M F	IP	Ca. 1	
	Adult	M F	PO	Ca. 0.5	
Pig	Weanling	M F	PO	Ca. 2	
Trout	100 g	M F	PO	Ca. 0.5	(8)

[a] Oral
[b] Intraperitoneal

values in the same order of magnitude as the duckling (Table IV). This is also true for the rainbow trout (8).

The relative lethal potencies of the four aflatoxins in the day-old duckling have been examined by Carnaghan *et al.* (20). The oral 7-day LD_{50} reported for each compound were aflatoxin B_1 18.2 μg, B_2 84.8 μg, G_1 39.2 μg, and G_2, 172.5 μg, all on a 50-gram body weight basis. These values illustrate clearly the relationships of structural configuration to acute lethality. Aflatoxin B_1 is most potent, followed by G_1, B_2, and G_2 in order of decreasing potency.

The toxic effects of aflatoxin B_1 have also been investigated in several in vitro cell culture systems and in embryonated eggs. These experimental systems are much more sensitive than in vivo systems in terms of the amounts of toxin required to produce effects. The early studies of Juhasz and Greczi (42) demonstrated that methanol extracts of peanut meals containing aflatoxins were toxic to calf kidney cells. No estimation of the aflatoxin concentrations was given. Legator and Withrow (49) have shown that very small concentrations (0.03 μg per ml.) of aflatoxin B_1 significantly inhibit the mitotic process in human embryonic lung cells.

Platt *et al.* (63) observed that aflatoxin mixtures were toxic to chick embryos, and this observation has been amply confirmed and extended

with aflatoxin B_1 The data of Verrett *et al* (*82*) indicate the sensitivity of the chick embryo, in which the LD_{50} is 0 048 μg per egg when administration is made via the yolk, and 0 025 μg per egg when the compound is applied in the air cell In both cases, the fertilized eggs were treated prior to incubation

Although they have not yet been fully exploited, the great sensitivity of cell cultures and embryos to the toxic effects of aflatoxin makes them potentially important bioassay systems for detection of low-level contamination of foodstuffs The relative lack of specificity of the embryo lethality response, however, requires that the test materials be available in highly purified form

In all species except the sheep, animals which consume sublethal quantities of the compounds for several days or weeks develop a subacute toxicity syndrome which commonly includes moderate to severe liver damage as a prominent pathologic sign The development of biliary hyperplasia represents the most consistently observed lesion The extent and severity of this histopathologic change can be semiquantitatively evaluated by the pathologist and has been used as a criterion of aflatoxin activity, particularly in the young duckling (*86*) This response provided the basis for biological assays for aflatoxin contamination of foodstuffs (*6*, *59*) and the response has been studied in some detail (*17* *58*, *88*) When the toxin is administered repeatedly to the day-old duckling over a 5-day period, characteristic lesions are observed on the seventh day Detailed examination (*86*) of the quantitative aspects of this response has revealed that the administration of aflatoxin B_1 to ducklings according to this dose schedule results in reproducible responses at a dose level of 0 5 μg per day (approximately 2 5 μg total dose) This level of sensitivity has made the assay useful in detecting contamination of foodstuffs

Subacute toxic effects of the aflatoxins in monkeys have been reported by Tulpule *et al* (*75*) In these experiments, young (1 5 to 2 0 kg) Rhesus monkeys were fed either 1 0 mg of aflatoxin per day or 0 5 mg per day for 18 days, then 1 0 mg per day thereafter All animals developed anorexia and died in 14 to 28 days The principal histopathologic findings included liver lesions similar to those seen in ducklings (portal inflammation and fatty change), suggestive of biliary cirrhosis In these experiments, the animals received a total dose of 10 to 15 mg per kg of body weight of a preparation containing 60% aflatoxin B_1 and 40% G_1 This report comprises the only published information available to date concerning the effects of the compounds in

The acute p[illegible] the aflato[illegible] established by the effects described [illegible] signific[illegible] from prolonged administration of [illegible] of [illegible] to animals In early [illegible] cont[illegible] meals, investigators

at the Unilever Research Laboratories in England (*47*) fed diets containing highly toxic peanut meals to rats After 6 months' feeding of 20% peanut meal (aflatoxin content not stated) in a purified diet, 9 of 11 rats developed multiple liver tumors, and two had developed lung metastases This finding represented the first indication of the carcinogenic properties of aflatoxin-contaminated, toxic peanut meals and has since been amply confirmed (*12, 19, 48, 65, 69*) Subsequent investigations have been concerned with the demonstration that the aflatoxins were the responsible carcinogenic agents and with the determination of dose, duration of exposure, and other conditions for tumor induction by these compounds

Although precise dose response conditions have not yet been established, some information is available regarding the dose response relationships between tumor incidence in rats and aflatoxin content of contaminated peanut meals The results of studies on several such meals (*57*) demonstrate good correlation between liver tumor incidence and dietary aflatoxin content over the range of 0 06 to 1 8 p p m of aflatoxin The highest level resulted in more than 90% tumor incidence when fed over a period of 370 days The lowest level of aflatoxin detected (0 005 p p m) failed to induce liver tumors within a similar time period (384 days)

Mice appear to be relatively resistant to acute poisoning by the aflatoxins as evidenced by results of short term feeding of heavily contaminated peanut meals Platonow (*62*) studied effects of diets containing 15 30 or 80% of a peanut meal containing 4 5 p p m each of aflatoxins B_1 and G_1 and 0 6 p p m each of aflatoxins B_2 and G_2 These diets were fed to groups of mice (20 to 25 grams) for at least 3 months During this time no effects were noted on feed intake or body weight, and no mortality occurred Histopathologic examination revealed no significant changes in liver or other tissues studied

Recent experiments with purified aflatoxin preparations have indicated that continuous feeding is not required for hepatoma induction in rats In the studies of Barnes and Butler (*12*), rats were fed 1 75 p p m of aflatoxin (containing 80% aflatoxins with G_1 present in higher quantities than B_1) in the diet for 89 days, then returned to an aflatoxin-free diet All of three treated animals ultimately developed liver cancer more than 300 days following withdrawal

In somewhat similar experiments in our laboratories (*86*) we have administered an unfractionated mixture of partially purified aflatoxins (approximately 30% B_1 20% G_1) to rats by stomach tube Each animal was treated daily for 30 days then held without treatment for a further 10 months Animals which received the highest dose (150 μg per rat per day) had well-developed liver tumors 5 months following withdrawal of treatment Even those receiving the lowest dose studied (15 μg per rat per day) showed significant incidence of precancerous lesions which

probably would have progressed to tumors over a longer period of time. These data would indicate that continuous exposure to the compounds is not required for liver tumor induction.

On the basis of these preliminary data, it has been possible to estimate the effective dose of aflatoxin B_1 for the induction of liver tumors in rats. Butler (*18*) has estimated this dose to be in the order of 10 μg. per day. When this value is compared with similar estimates for other hepatocarcinogens such as dimethylnitrosamine (750 μg. per day) and butter yellow (9000 μg. per day), the relative potency of the compound is readily apparent.

Recent studies by Ashley *et al.* (*8, 9*) and by Sinnhuber *et al.* (*72*) have suggested that the rainbow trout may be considerably more sensitive than the rat to the hepatocarcinogenic effects of the aflatoxins. These investigators have shown that rainbow trout develop liver tumors at significant incidence rates when fed purified diets containing only 0.5 to 2.0 μg. of aflatoxin B_1 per kilogram—i.e., 0.5 to 2.0 p.p.b. The apparent sensitivity of this species has led to the recognition (*8*) of the potential role of the aflatoxins as etiologic agents in the so-called "trout hepatoma syndrome" (*33, 37*).

In a different test system, Dickens and Jones (*25*) studied the effects of multiple subcutaneous injections of aflatoxins B_1 and G_1 in rats. A mixed preparation (about 38% B_1 and 56% G_1) of the compounds dissolved in peanut oil was administered to groups of rats twice weekly. One group received 50 μg. at each injection, and the treatment was continued for 50 weeks; a second group received 500 μg. at each injection for only 8 weeks, after which treatment was discontinued. In the former group, 6/6 animals developed sarcomas or fibrosarcomas at the injection site within a 60-week period. At the higher dose level, 5/5 animals developed tumors within a 30-week period. These observations indicate that the compounds are also carcinogenic for the subcutaneous tissues of the rat.

Biochemical alterations caused by aflatoxins have been studied in a number of biological systems. Smith (*74*) investigated the effects of aflatoxin B_1 on the rate of in vitro incorporation of leucine-C^{14} into proteins by rat and duckling liver slices. Significant reduction in the rate of incorporation in 80 mg. of rat liver slices was caused by adding 10 μg. of toxin, and almost complete suppression was effected by 200 μg. Duckling liver slices studied under similar conditions were more sensitive, showing significant reduction with 3.5 μg. of toxin. These observations suggested that the compound suppresses protein synthesis by some mechanism, and this activity has been investigated in other test systems.

We have studied (*71*) the in vivo incorporation of leucine into rat liver proteins at time intervals following single sublethal doses of aflatoxin B_1. A single oral dose of 4.76 mg. of toxin per kg. of body weight, im-

mediately suppressed, then increased amino acid incorporation Inhibition was clearly evident within 30 minutes following dosing, and was maximal at 6 hours, at which time the rate of incorporation was reduced to approximately 50% of the control value This was followed by a 72-hour period during which the incorporation in the treated animals exceeded the control rate, being maximal 72 hours after toxin administration

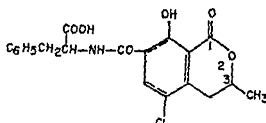
These results led to further investigation of effects on synthesis of specific proteins in which we have determined the influence of the toxin on liver tryptophan pyrrolase induction by hydrocortisone and by tryptophan in rats (*87*) The increase in activity of this enzyme in liver 6 hours following intraperitoneal injection of 150 mg of hydrocortisone per kg or 600 mg of tryptophan per kg was studied in weanling male rats Animals receiving aflatoxin B_1 at a level of 1 mg per kg and hydrocortisone showed no increase in enzyme activity 6 and 12 hours following toxin administration, as compared to a fourfold increase in those receiving hydrocortisone only Although this inhibitory effect was not apparent 5 days later, larger doses (3 or 5 mg per kg) of aflatoxin caused inhibition of enzyme induction which persisted for at least 10 days Animals treated with 5 mg of toxin per kg showed no significant inhibition of enzyme induction by tryptophan The inhibition of hydrocortisone induction, which involves *de novo* enzyme synthesis and the failure to inhibit tryptophan induction which depends upon enzyme stabilization without synthesis suggests that the toxin is an inhibitor of protein synthesis at some specific stage The locus of this effect is currently under investigation

Black and Altschul (*13*) have recently reported finding that gibberellic acid induced increases in lipase and α amylase activity of the germinating cottonseed are inhibited by aflatoxin This effect is similar qualitatively to the inhibition of enzyme induction in rat liver described above although it is not yet clear whether the mechanism of inhibition is related in the two systems

The aflatoxins also have interesting effects in tissues of plant origin Schoental and White (*70*) have shown that aflatoxins in concentrations of 25 μg per ml inhibit the germination of the seeds of cress (*Lepidium sativum*, L) Smaller concentrations apparently interfered with chlorophyll synthesis since there was complete absence of color when the germinating seeds were exposed to the compounds in concentrations of 10 μg per ml

The rapid development of knowledge concerning the aflatoxin problem has stimulated new research activities in the mycotoxin field, some of which have already begun to yield results For example workers in South Africa (*79*) have recently isolated and chemically characterized a new compound, ochratoxin A

This compound was isolated from cultures of *Aspergillus ochraceus*, an organism known to invade stored wheat and has an LD_{50} in ducklings



similar to that of aflatoxin B_1 It is virtually certain that, as the search is intensified, additional toxic fungal metabolites will be discovered

Conclusions

The background of information summarized here illustrates the range and diversity of problems which can arise from contamination of food stuffs by mycotoxins Utilization of world food resources with maximum efficiency and safety therefore will require the development of agricultural and processing methods which will prevent mycotoxin contamination or permit detection and removal of contaminants when present These factors will be of particular importance to the exploitation of new foods for human use

Acknowledgment

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On the basis of these preliminary data, it has been possible to estimate the effective dose of aflatoxin B_1 for the induction of liver tumors in rats Butler (*18*) has estimated this dose to be in the order of 10 μg per day When this value is compared with similar estimates for other hepato carcinogens such as dimethylnitrosamine (750 μg per day) and butter yellow (9000 μg per day), the relative potency of the compound is readily apparent

Recent studies by Ashley *et al* (*8, 9*) and by Sinnhuber *et al* (*72*) have suggested that the rainbow trout may be considerably more sensitive than the rat to the hepatocarcinogenic effects of the aflatoxins These investigators have shown that rainbow trout develop liver tumors at significant incidence rates when fed purified diets containing only 0 5 to 2 0 μg of aflatoxin B_1 per kilogram—i e , 0 5 to 2 0 p p b The apparent sensitivity of this species has led to the recognition (*8*) of the potential role of the aflatoxins as etiologic agents in the so-called "trout hepatoma syndrome" (*33, 37*)

In a different test system, Dickens and Jones (*25*) studied the effects of multiple subcutaneous injections of aflatoxins B_1 and G_1 in rats A mixed preparation (about 38% B_1 and 56% G_1) of the compounds dissolved in peanut oil was administered to groups of rats twice weekly One group received 50 μg at each injection, and the treatment was continued for 50 weeks, a second group received 500 μg at each injection for only 8 weeks, after which treatment was discontinued In the former group, 6/6 animals developed sarcomas or fibrosarcomas at the injection site within a 60-week period At the higher dose level, 5/5 animals developed tumors within a 30 week period These observations indicate that the compounds are also carcinogenic for the subcutaneous tissues of the rat

Biochemical alterations caused by aflatoxins have been studied in a number of biological systems Smith (*74*) investigated the effects of aflatoxin B_1 on the rate of in vitro incorporation of leucine-C^{14} into proteins by rat and duckling liver slices Significant reduction in the rate of incorporation in 80 mg of rat liver slices was caused by adding 10 μg of toxin, and almost complete suppression was effected by 200 μg Duckling liver slices studied under similar conditions were more sensitive, showing significant reduction with 3 5 μg of toxin These observations suggested that the compound suppresses protein synthesis by some mechanism, and this activity has been investigated in other test systems

We have studied (*71*) the in vivo incorporation of leucine into rat liver proteins at time intervals following single sublethal doses of aflatoxin B_1 A single oral dose of 4 76 mg of toxin per kg of body weight, im

mediately suppressed then increased amino acid incorporation Inhibition was clearly evident within 30 minutes following dosing, and was maximal at 6 hours, at which time the rate of incorporation was reduced to approximately 50% of the control value This was followed by a 72-hour period during which the incorporation in the treated animals exceeded the control rate being maximal 72 hours after toxin administration

These results led to further investigation of effects on synthesis of specific proteins in which we have determined the influence of the toxin on liver tryptophan pyrrolase induction by hydrocortisone and by tryptophan in rats (*87*) The increase in activity of this enzyme in liver 6 hours following intraperitoneal injection of 150 mg of hydrocortisone per kg or 600 mg of tryptophan per kg was studied in weanling male rats Animals receiving aflatoxin B_1 at a level of 1 mg per kg and hydrocortisone *showed no increase in enzyme activity 6 and 12 hours following* toxin administration, as compared to a fourfold increase in those receiving hydrocortisone only Although this inhibitory effect was not apparent 5 days later larger doses (3 or 5 mg per kg) of aflatoxin caused inhibition of enzyme induction which persisted for at least 10 days Animals treated with 5 mg of toxin per kg showed no significant inhibition of enzyme induction by tryptophan The inhibition of hydrocortisone induction, which involves *de novo* enzyme synthesis and the failure to inhibit tryptophan induction which depends upon enzyme stabilization without synthesis suggests that the toxin is an inhibitor of protein synthesis at some specific stage The locus of this effect is currently under investigation

Black and Altschul (*18*) have recently reported finding that gibberellic acid induced increases in lipase and α amylase activity of the germinating cottonseed are inhibited by aflatoxin This effect is similar qualitatively to the inhibition of enzyme induction in rat liver described above although it is not yet clear whether the mechanism of inhibition is related in the two systems

The aflatoxins also have interesting effects in tissues of plant origin Schoental and White (*70*) have shown that aflatoxins in concentrations of 25 μg per ml inhibit the germination of the seeds of cress (*Lepidium sativum* L) Smaller concentrations apparently interfered with chlorophyll synthesis since there was complete absence of color when the germinating seeds were exposed to the compounds in concentrations of 10 μg per ml

The rapid development of knowledge concerning the aflatoxin problem has stimulated new research activities in the mycotoxin field, some of which have already begun to yield results For example workers in South Africa (*79*) have recently isolated and chemically characterized a new compound ochratoxin A

This compound was isolated from cultures of *Aspergillus ochraceus*, an organism known to invade stored wheat and has an LD_{50} in ducklings

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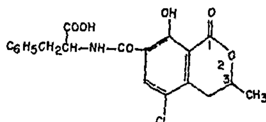
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similar to that of aflatoxin B_1 It is virtually certain that, as the search is intensified, additional toxic fungal metabolites will be discovered

Conclusions

The background of information summarized here illustrates the range and diversity of problems which can arise from contamination of food stuffs by mycotoxins Utilization of world food resources with maximum efficiency and safety therefore will require the development of agricultural and processing methods which will prevent mycotoxin contamination or permit detection and removal of contaminants when present These factors will be of particular importance to the exploitation of new foods for human use

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The contributions of P M Newberne, R C Shank, and M M Abdel Kader to these studies are gratefully acknowledged

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15

Some Approaches to the Elimination of Aflatoxin From Protein Concentrates

LEO A GOLDBLATT

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Three approaches to eliminating aflatoxin from protein concentrates are prevention, removal, and inactivation The best approach is prevention Mechanical damage should be avoided during harvesting and handling, and moisture, temperature, and infestation with insects should be controlled during storage Mechanically removing the contaminated kernels or seeds may be effective Alternatively, removing aflatoxin with selective solvents presents good potential Polar solvents can be used to remove aflatoxin from oilseeds during processing to oil and meal or from the finished protein concentrates Aflatoxin must be inactivated without destroying nutritive value or leaving toxic residues Exposure to moist heat and treatment with gaseous reagents such as ammonia, or with reagents added during processing, may offer practical means of detoxification

Aflatoxin is the product of a mold Molds and mold damage have long been of interest to those concerned with agricultural products because of economic losses due to obvious decay and more insidious adverse changes in the odor, taste, appearance, and nutritive value, and because, mold causes seeds to lose their viability, making them unsuitable for planting Much research has been done to find ways to minimize such losses Most of this research has been directed toward practical methods of preventing or minimizing mold growth by determining at which stages mold develops during the growth, harvesting, and subsequent handling of a commodity and which conditions at these stages favor or inhibit the growth of molds In consequence, although precise information may be lacking, we know how to reduce molds sharply When the aflatoxin

problem was recognized, the importance of this knowledge increased since much of the information already developed was relevant to minimizing the occurrence of this specific fungal metabolite in agricultural products

Prevention

All molds do not produce aflatoxin, and even growth of aflatoxin-producing molds is not necessarily accompanied by production of aflatoxin In general, however a product for human consumption should not be prepared from damaged seeds or from seeds of bad cultural history Prevention, then, comprises the first approach Much of my discussion of prevention is drawn from a report recently issued by the Agricultural Research Service United States Department of Agriculture (*24*)

Modern farming know how and equipment make it possible to keep mold levels to a minimum Although procedures recommended for preventing mold damage vary with the crop its stage of maturity, and environmental conditions certain broad principles apply to most oilseed and grain crops Mold prevention begins on the farm with properly planting and growing the crop There is much evidence that sound, healthy seed is more resistant to mold growth and production of aflatoxin than damaged seed Using sound, fungus free viable seed, fertilizing properly and controlling insects and diseases not only increase vigor of the young plants but also reduce the likelihood that mold will become established in the plant at maturity

Although we do not know enough about how specific cultural practices during crop growth affect the later development of mold in a crop we know that harvesting and handling phases are critical In harvesting, equipment should be adjusted and operated to avoid mechanical damage Machinery now used may have to be slowed down or even redesigned Harvesting promptly at maturity is generally to be recommended Permitting the crop to stand in the field after ripening is an invitation to invasion by insects and mold Oilseed and grain crops should be cleaned thoroughly to remove foreign matter and damaged seed, and storage areas should be weather tight clean and free of insects Kernels attacked by insects are especially susceptible to invasion by molds

Moisture control is crucial in mold prevention Moisture content is higher in immature crops Warm temperatures also encourage mold, especially in combination with high relative humidity Consequently it is important to dry the commodity to a safe moisture level as soon after harvesting as practicable and maintain it at that level For this proper aeration is required Without adequate aeration, significant differences in temperature between different locations in stored material may build up causing the moisture to concentrate to damaging levels in the colder

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Moisture control is crucial in mold prevention Moisture content is higher in immature crops Warm temperatures also encourage mold especially in combination with high relative humidity Consequently it is important to dry the commodity to a safe moisture level as soon after harvesting as practicable and maintain it at that level For this, proper aeration is required Without adequate aeration significant differences in temperature between different locations in stored material may build up, causing the moisture to concentrate to damaging levels in the colder

spots and resulting in serious mold growth, even when the average moisture content would appear to be at a safe level Mold produces moisture as it grows, so once fungal growth has started in one excessively wet kernel the moisture content of adjacent kernels increases and further fungal proliferation may proceed, resulting in a pocket of highly contaminated material Accordingly, adequate sampling and testing are a must

Peanuts Damaged peanuts are particularly susceptible to mold Damage prevention begins with effective pod-insect and disease control during the growing period and continues through harvest

Peanuts are more like green vegetables than most oil and cereal crops in the care they require in harvest, handling, and storage to prevent mold damage When dug, peanuts usually have an average moisture content between 35 and 55%, with individual seeds on the same plant ranging from below 30 to as much as 70% moisture For safe storage, the moisture must be reduced to about 8% Combines pick up some earth and foreign matter, which should be removed promptly Machinery should be operated so that the peanuts are not damaged—for example, by operation at excessive speed

Cottonseed In the past, concern over *Aspergillus flavus* in growing cotton centered on the fact that the organism yellows and downgrades the lint Mold in cottonseed was also known to increase the free fatty acids in the oil and thus reduce its value We know that some strains of *A flavus* can produce aflatoxin in cottonseed Overfertilization and overirrigation promote rank growth and boll rot, which is caused by some molds, in the lower portion of the plant Bottom defoliation when these lower bolls are mature prevents boll rot Cotton should be harvested as soon after opening as is economically feasible, but not when the cotton is damp Mechanical cotton pickers and gins should be operated to prevent or minimize seed cracking Moisture should be reduced as promptly as possible If moisture in the seed cotton is high or large amounts of green leaves or other trash are present, the cotton may deteriorate and mold begin to grow within 24 hours Cottonseed with about 10% moisture content has been stored without significant damage

Soybeans With soybeans as with other oilseeds and small grains, avoiding seed damage and reducing the moisture content to safe levels promptly after harvest are important to prevent mold Combines should be operated at a speed that does not crack the seed Since the moisture content of the field may change during the day, it may be necessary to adjust the combine periodically to reduce splits and other damage

Removal

The potential for molds and possibility of toxicity of mold metabolites accent a long-standing concern for quality Advances in basic knowledge

and in techniques for growing harvesting, and handling crops will make it easier to produce and market commodities free of mold damage However, damage may occur despite the most strenuous efforts directed at prevention Hence two other approaches must be considered—removal and inactivation

Let us examine first the potential for removal Two approaches may be considered physical removal or culling of contaminated from sound seed or kernels and removal of the aflatoxin

Removal of Contaminated Kernels or Seed The fortuitous circumstance that the vast majority of the aflatoxin in contaminated materials may reside in a relatively small number of seeds affords an exceptional opportunity for effectively yet economically reducing the aflatoxin content of selected materials by mechanically removing contaminated seeds In the case of peanuts even in rare lots having relatively large amounts of aflatoxin—and we have diligently sought such lots for investigation—only a very small percentage of the kernels contain aflatoxin For example, we have found a number of peanuts with more than 100,000 parts per billion of aflatoxin and a few with more than a million p p b If just one peanut with a million p p b of aflatoxin is admixed with 10 000 kernels that contain none the whole lot will assay at the relatively high level of 100 p p b of aflatoxin Although low incidence of contaminated kernels makes sampling very difficult it affords an excellent opportunity for economical culling

The peanut industry has recognized that culling on the basis of physical characteristics such as size (including immatures) obvious damage and discoloration appears feasible and has taken steps to improve mechanical separation Electronic sorters seem to be very effective In a series of experimental runs it was demonstrated conclusively that suspect material can be removed from raw peanuts by electronic and manual picking procedures although further improvement resulted when the picking procedures were repeated following roasting and blanching (*13, 17*) Segregation after splitting peanut kernels may be necessary to eliminate aflatoxin resulting from mold invasion of the interior not manifest on the surface Also, peanut kernels invaded by mold between the two cotyledons appear to be distinctly harder to split than sound kernels The mold or mycelium seems almost to cement the two cotyledons together Careful control of the splitting operation and removal of the whole kernels may offer a practical way to cull kernels with hidden mold damage

In the case of cottonseed segregation on the basis of density may be possible A projection device to separate infested from noninfested grain kernels had been described by Katz, Farrell, and Milner (*12*) Several years ago various projection devices such as the ARS differentiator and the cottonseed cleaning belt unit were developed at the Southern Utilization Research and Development Division and operated on a pilot-plant

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scale to separate trash from cottonseed These devices graded cottonseed according to density and other characteristics The bulk of the foreign matter and the very light immature and decayed seed collect relatively close to the projector, and the seeds that project further are of higher quality—i e, higher in oil and nitrogen content and lower in free fatty acid in the oil (*9*)

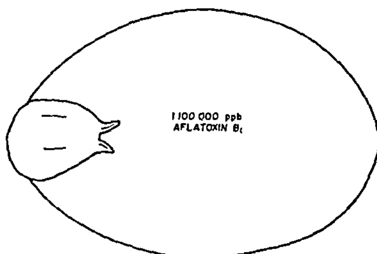
A high correlation has been reported (*6*) between fat acidity and incidence of aflatoxin, at least in the case of peanuts

Relation of Aflatoxin to Fat Acidity in Peanuts

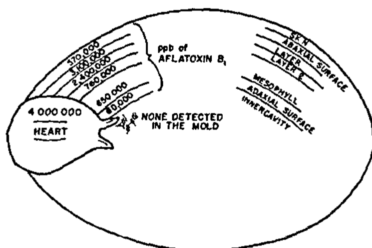
Fat Acidity	*% Samples Containing Aflatoxin*
< 0 3	8
0 31–1 0	30
> 1 0	70

Accordingly, since high free fatty acid in fresh cottonseed oil is associated with mold damage, such a device may afford a means of mechanically removing all or major portions of aflatoxin-contaminated cottonseed When this hypothesis was tested experimentally, a marked separation was obtained, some portions contained relatively large amounts of aflatoxin, whereas other portions contained relatively little or none Classification of cottonseed on an air table is an effective way of selecting cottonseed to give maximum emergence after planting, the denser seed giving the higher emergence (*11*) Impermeable seed coat cottonseed, so-called "hard seed," has been reported to develop much less fat acidity than "soft" cottonseed stored under the same conditions (*4*) Thus, the possibility exists of controlling mold invasion, and hence production of aflatoxin in cottonseed, by genetic means The Crops Research Division, U S Department of Agriculture, in cooperation with several state agricultural experiment stations, has undertaken long-range research to investigate genetic resistance and the way it is inherited and to breed varieties of peanuts with pods and seeds relatively more resistant to molds that produce toxins

Removal of Aflatoxin from Contaminated Kernels or Seed The feasibility of removing aflatoxin by a simple washing or "laundering" operation—for example, washing whole peanut kernels with water or dilute alkali—has been the object of much discussion The aflatoxin contents of different parts of peanut kernels containing large amounts of aflatoxin can be determined (*5*) In Figure 1 is illustrated one cotyledon of such a peanut kernel The other cotyledon of this kernel, not illustrated, contained more than a million parts per billion of aflatoxin Thin layers were carefully sliced from the curved surface of the half kernel shown in Figure 1 Other selected portions were completely excised with a razor blade and various portions were analyzed separately (*5*) The


1 100 000 ppb
AFLATOXIN B_1

COTYLEDON A


370 000
3,100,000
2,400,000
760 000
650 000
80,000
ppb of
AFLATOXIN B_1
4 000 000
HEART
NONE DETECTED
IN THE MOLD
SKIN
ABAXIAL SURFACE
LAYER
LAYER
MESOPHYLL
ADAXIAL SURFACE
INNERCAVITY

hexane and water may be a practical system in processing peanuts to oil and aflatoxin free meal This solvent mixture could also be used to remove aflatoxin from meals or protein concentrates produced by other means such as pressing or solvent extraction with hexane A major advantage is that it removes relatively little extraneous material

Another opportunity is offered by a solvent such as aqueous acetone The solvent currently used to determine gossypol is 70% acetone (*30 volumes of water 70 volumes of acetone*) *It readily dissolves afla-*toxin and is the basis for an analytical procedure for determining aflatoxin in cottonseed products (*19*) Since glyceride oils are relatively insoluble in 70% acetone it is a selective solvent whereby aflatoxin may be removed from prepared peanuts or cottonseed meats The residue can be processed to a full fat product or partially defatted by pressing or solvent extraction In the case of cottonseed this solvent leaves a full fat gossypol free product The potentially useful solvent systems that may be considered are limited only by imagination and economic justification

The efficiencies of different aqueous salt solutions in extracting aflatoxin from ground peanut meals or pastes has been reported (*22*) Extraction with 1% $NaHCO_3$ completely removed aflatoxin but also extracted 33% of the protein One per cent $CaCl_2$ solution reported to be the most promising removed 80% of the toxin but only 10% of the protein In preparing protein isolates precipitation of the protein in $CaCl_2$ solution removed nearly 80% of the toxin from the protein

Inactivation

Finally there is the third approach treatment to destroy or inactivate aflatoxin However, any such treatments must not only inactivate aflatoxin but also leave unimpaired the nutritive value of the material processed and leave no deleterious residues that might result from molds Numerous treatments have been proposed but relatively few are reported in the literature, much of what is reported is sketchy and contradictory, and little is said about the nutritive value of the treated product

The possibility of destroying aflatoxin by gamma radiation is a subject of frequent speculation Kraybill and Shimkin (*16*) have reported that preliminary unpublished work of British workers indicates that even a 2 megarad dose of radiation did not destroy aflatoxin No published reports are known relating to the use of microwaves to inactivate aflatoxins, but several groups including some in the department, plan to investigate this possibility

The effect of heat has been studied Fischbach and Campbell (*7*) reported that it was necessary to raise the temperature to 300° C or above to decompose aflatoxins Blount (*3*) reported that heating peanut meal to 150° C for one hour appeared to make the product even more

toxic (for turkey poults), but Allcroft (*1*) reported that toxicity of the meal to ducklings was always less after it was heated Another publication states that when toxic meal was heated to 150° C for several hours, the protein was denatured but the toxicity was not reduced (*2*) A more recent report (*20*) stated that sterilization (at 15 p s i steam for 4 hours) reduced the aflatoxin content of a highly toxic meal to relatively harmless levels (from 7000 to about 1000 μg per kg in 4 hours) Our own unpublished results indicate that aflatoxin content can be decreased by heating at moderate temperatures and that the moisture content of the substrate has a significant effect This variable may account for some of the apparently conflicting reports in the literature

From results of trials run in an attempt to find a simple method of removing or inactivating aflatoxin in batches of affected peanut meal, it was concluded (*10*) that "heat treatment, and exposure to acid, alkali, chlorine, sulfur dioxide, gamma-radiation, and ultraviolet light were all largely ineffective" The toxin appeared to be "protected" by physical barriers in the meal, and it was difficult to attack it without seriously impairing the nutritive and other properties of the meal Recently Fischbach and Campbell (*7*) reported that aflatoxin extracts treated for a few seconds with about 5% NaOCl lost their fluorescence, and the reaction products were nontoxic when subjected to chick embryo bioassay They also reported that a highly contaminated peanut meal (about 1000 p p b) exposed overnight to a 10% chlorine gas atmosphere lost about 90% of its initial fluorescence and became nontoxic to the chick embryo They noted that several oxidizing agents eliminated or drastically reduced fluorescence of aflatoxins, and that a number of oxidizing agents are commonly used in food processing, for bleaching flour, and in some fumigation procedures They are currently exploring the effectiveness of a variety of oxidizing agents

The potential of alkalies applied at various stages of processing oil seeds to oil and meal has been the subject of much speculation and some investigation Such treatment is attractive from the standpoint of economy and ease of introduction into currently used processes *A priori*, alkalies would be expected to react readily with the lactone moiety of the aflatoxins and it is known that aqueous alkalies readily remove aflatoxin from crude oil Reports received in various personal communications have been conflicting but generally indicated reduction of aflatoxin content when relatively small quantities of alkalies, such as NaOH or Na_2CO_3, are introduced at various stages of processing of cottonseed or peanuts to oil and meal Our own unpublished observations confirm that alkalies added during processing may afford an oilseed meal (protein concentrate) significantly lower in aflatoxin than the level calculated as the difference between the amount present in the cottonseed or peanuts used and that recovered in the oil

Treatment with ammonia or ammonium hydroxide has been reported to detoxify tung meal (25) and castor meal (8) Recently Masri reported (16) that treating a toxic peanut meal (about 1000 p p b aflatoxin B_1) with ammonia eliminated the toxicity Cooperative work at the Southern Regional Research Laboratory has confirmed and extended this significant finding Accordingly, it appears that alkali treatment of oilseeds during processing or of the finished protein concentrate offers a promising approach to the elimination of aflatoxin

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Discussion

Bernard L Oser Dr Goldblatt has pointed out that of the several alternatives—prevention, removal, or inactivation of aflatoxin—the first was the consummation most devoutly to be wished Even if removal or inactivation were feasible, these procedures might have little possibility of application in the United States, where the attitude of the Food and Drug Administration (FDA) is opposed to treating or reconditioning an adulterated food to make it conform with the sanitary requirements for wholesome foods

Furthermore in the case of aflatoxin, we are dealing with a substance whose carcinogenicity has been well established in several species of animals Whether the prohibition under the Delaney clause is applicable in this instance or can be successfully avoided on the ground that the toxic mold is not 'added' is a moot question For example would FDA permit the reconditioning of botulinum infected food? Aflatoxin is in the same order of magnitude as a toxic agent

If removal or inactivation processes are not *100% effective* the problem of "zero tolerance ' would still remain, in which connection the recent

report of the Pesticide Residue Committee of the National Academy of Sciences/National Research Council is relevant

It is important to bear in mind the impact of our Government's standard of purity and quality of food on the attitude of other countries, even in so-called underdeveloped areas of the world Why should a country import a food product which according to U S standards is considered adulterated? The positions taken by the FDA with respect to whole fish flour as a food for man or the supplementation of wheat flour with lysine to improve its protein quality are cases in point The view that these are not permitted or not needed certainly tends to discourage technological research in their respective fields

16

Better Protein Quality in Maize

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Maize endosperm homozygous for the opaque-2 mutant gene has a different protein and amino acid composition than normal maize endosperm Both lysine and tryptophan are increased The increased lysine content in the endosperm is due to increased lysine in the acid-soluble fraction and in the zein fraction and reduction in the ratio of zein to glutelin Feeding tests with young albino rats show that opaque-2 maize proteins are superior to the proteins of any cultivated cereal grain and have a protein efficiency ratio equal to that of heat-treated soybean meal The opaque-2 gene exerts its effect in a high protein background A second mutant gene, floury-2, also increases the lysine and, in addition, the methionine content of normal maize endosperm

For optimum performance, corn is never fed as the sole source of protein in animal rations because of its relatively low protein content and poor balance of essential amino acids Howe, Jansen, and Gilfillan (*12*) discuss the limiting amino acids in corn and other cereals as determined by feeding tests in animals An excellent demonstration of its palatability and source of many nutrients was made 12 years ago, when we devised a special diet to determine the amino acid requirements of swine (*1*) The ration in Table I was fed to 5- to 6-week-old crossbred barrows averaging 24 3 pounds They gained an average of 1 pound a day for 35 days, with a protein efficiency of 3 1 pounds of gain per pound of crude protein This efficiency is greater than the values of 2 1 to 2 6 obtained with high quality natural proteins (*1*) In this diet (Table I), corn containing 8 2% crude protein (N × 6 25) was supplemented with a mixture of the ten amino acids found in earlier studies to be essential for the weanling pig

These data show that corn is a good source of nitrogen, carbohydrate, and lipids, and needs only to be supplemented with minerals, vitamins,

and certain amino acids to become a complete feed A level of 18% protein is usually recommended for the 25 pound weanling pig on a diet of natural feedstuffs The diet in Table I contains only 13 2% crude protein, yet is more effective than the natural diet with a higher protein content

Improving Quality of Corn Proteins by Breeding

The amino acid pattern of whole corn can be changed by breeding for larger germ size in the kernel (*7, 8*) The protein content of the endosperm can be increased by selective breeding and nitrogen fertilization (*10, 11*) Unfortunately, the zein content usually increases with increases in nitrogen Recently Telle *et al* in Mexico City (*30*) determined the lysine content of 182 varieties of corn and found mean values ranging from 260 to 364 mg per 100 grams of whole kernel The average value of two standard Indiana hybrids harvested in 1964 was 300 mg per 100 grams of whole kernel (*23 24*) Telle *et al* believe that lysine content is a racial characteristic and plan to select specimens for breeding work leading to more nutritious corn hybrids

Table I Experimental Corn–Amino Acid Diet[a]

Component	*% of Ration*
Corn	88 00
Ten amino acids	6 44
Diammonium citrate	0 57
Minerals	4 30
Liver extract	0 50
Cerelose	0 19
	100 00

[a] Corn contained 8 2% crude protein 14 1% moisture Vitamin supplement fed daily

Extraction and Fractionation of Corn Proteins

The classical Osborne method (*3 27, 28*) and its modifications (*5, 25*) utilize a dilute salt solution to remove albumins and globulins, an aqueous ethyl alcohol solution to extract zein, and a dilute alkali solution to dissolve the glutelins in maize endosperm The methods are time-consuming and in our hands have never resulted in a recovery of more than 85% of the total nitrogen

In 1957, we developed a copper reagent (Table II) that extracted 89 to 98% of the nitrogen from the defatted endo perm of 11 varieties and 89 to 100% of the nitrogen from the defatted germ of six varieties (*2, 20, 21*) The copper extract was adjusted to pH 3 to precipitate the zein and glutelin

These fractions may not be identical with the classical zein and glutelin fractions described by Osborne. Mertz and Bressani (*20*) discuss possible differences.

The supernate contains acid-soluble proteins and was labeled "acid soluble fraction." The precipitate was dissolved in 66% ethanol at pH 11.5. At this pH the glutelins as well as zein are soluble. The glutelins were then precipitated from solution by dropping the pH to 6.0, leaving the zein in the supernate. This permitted recovery of the three fractions described in Table II.

When the endosperms of several varieties of corn (*Zea mays*) were extracted and fractionated, the distribution of nitrogen among the acid soluble, zein, and glutelin fractions closely resembled that obtained with saline, ethanol, and alkali extractions in the classical Osborne method (*2, 21*). The acid-soluble fraction varied from 16 to 26, the zein from 41 to 60, and the glutelin from 17 to 31% of the endosperm nitrogen (Table I).

Table II. Protein Fractions from Copper Extract[a]

Fraction	*Properties*	*Total Nitrogen* Endosperm	Germ
Residue	Insoluble in reagent	2–11	0–11
Acid-soluble	Not precipitated from extract at pH 3	16–26	30–40
Zein	Precipitated at pH 3, soluble ethanol pH 6	41–60	5–10
Glutelin	Precipitated at pH 3, insoluble ethanol pH 6	17–31	49–54

[a] Copper reagent, 0.4% $CuSO_4 \cdot 5H_2O$, 0.05% Na_2SO_3, 0.2% NaOH.

Chemical Nature of Corn Proteins

Most of the work on corn proteins has been confined to the zein and glutelin components of the endosperm. Zein from endosperm is soluble in neutral (aqueous) ethanol and is therefore classed as a prolamine. Purified zein is a heterogeneous protein with molecules having molecular weights in the range of 20,000 to 50,000 (*14*). The molecules are long, with an axial ratio of about 20 to 1 (*9*).

Glutelin is soluble in alkali and in alkaline ethanol (*21*) but insoluble in neutral (aqueous) ethanol. Purified glutelin is a heterogeneous protein with molecules ranging in weight from 20,000 to more than 1,000,000 (*15*). Like zein, the smaller molecules have an axial ratio of about 20 to 1 (*15*).

Since lysine is the most limiting essential amino acid in corn, it is of interest to compare the lysine content of various fractions obtained by the copper method (*2*) The acid soluble and "glutelin" fractions of germ contain 6 4 and 5 8% of lysine in the proteins, respectively, and these two fractions together represent 90 to 95% of the nitrogen (*2*) The acid soluble and glutelin fractions of the endosperm contain 2 9 and 2 2% of lysine in the proteins respectively Thus, the acid soluble and glutelin fractions of the endosperm contain less lysine than the corresponding fractions of the germ These data represent values from composite samples but nevertheless should indicate comparative values among the fractions As expected the zein of endosperm contained the lowest level of lysine found in any fraction (0 08%)

Mutant Gene Changing Protein Composition of Endosperm

With the development of the copper extraction fractionation method for separating maize proteins (*2 15 20 21*) we searched for maize with a lower zein and a higher lysine content The importance of examining the separated endosperms was recognized (*16*) In 1963, a survey of mutants suspected of having blocks in zein production showed that a strain homozygous for the recessive mutant gene, *opaque 2* (o), had in the endosperm a lysine content (4% of the protein) twice that found in typical hybrid corn (*18*) (Unless indicated otherwise all amino acid analyses were made with a Spinco automatic amino acid analyzer)

Copper fractionation (*21*) of protein from *opaque 2* endosperm revealed 15 7% zein and 42 3% glutelin based on total protein (*17*) Endosperms from normal North American and Guatemalan maize lines (*2*) contained 41 to 52% zein and 17 to 28% glutelin on the same basis Thus, there is a reversal in the ratio of zein to glutelin in the *opaque-2* endosperm when compared with normal maize lines Two additional strains containing the *opaque 2* gene in different genetic backgrounds from each other, and from the *opaque 2* first tested also had lysine levels twice that found in normal endosperms

To make a critical test of the hypothesis that the *opaque 2* mutant is responsible for the increased lysine content, the normal and *opaque 2* kernels from a single backcross ear ($+/o_2 \times o_2/o_2$) were separated (*18*) The amino acid composition of the defatted acid hydrolyzed endosperms is shown in Table III Both types of endosperms contain 8 69% crude protein (N × 6 25) on a fat and moisture free basis Thus, the amino acid contents are directly comparable

The *opaque 2* endosperm (Table III) contains 69% more lysine than the normal endosperm The former contains less glutamic acid, alanine, leucine and tyrosine and more lysine, histidine arginine, aspartic acid, and glycine, than the latter These changes have been confirmed in

These fractions may not be identical with the classical zein and glutelin fractions described by Osborne Mertz and Bressani (*20*) discuss possible differences

The supernate contains acid-soluble proteins and was labeled "acid soluble fraction" The precipitate was dissolved in 66% ethanol at pH 11 5 At this pH the glutelins as well as zein are soluble The glutelins were then precipitated from solution by dropping the pH to 6 0, leaving the zein in the supernate This permitted recovery of the three fractions described in Table II

When the endosperms of several varieties of corn (*Zea mays*) were extracted and fractionated, the distribution of nitrogen among the acid soluble, zein, and glutelin fractions closely resembled that obtained with saline, ethanol, and alkali extractions in the classical Osborne method (*2*, *21*) The acid-soluble fraction varied from 16 to 26, the zein from 41 to 60, and the glutelin from 17 to 31% of the endosperm nitrogen (Table I)

Table II Protein Fractions from Copper Extract[a]

Fraction	*Properties*	*Total Nitrogen* *Endosperm*	*Germ, %*
Residue	Insoluble in reagent	2–11	0–11
Acid-soluble	Not precipitated from extract at pH 3	16–26	30–40
Zein	Precipitated at pH 3, soluble ethanol, pH 6	41–60	5–10
Glutelin	Precipitated at pH 3 insoluble ethanol, pH 6	17–31	49–54

[a] Copper reagent 0 4% $CuSO_4$ $5H_2O$ 0 05% Na_2SO_3 0 2% NaOH

Chemical Nature of Corn Proteins

Most of the work on corn proteins has been confined to the zein and glutelin components of the endosperm Zein from endosperm is soluble in neutral (aqueous) ethanol and is therefore classed as a prolamine Purified zein is a heterogeneous protein with molecules having molecular weights in the range of 20 000 to 50,000 (*14*) The molecules are long, with an axial ratio of about 20 to 1 (*9*)

Glutelin is soluble in alkali and in alkaline ethanol (*21*) but insoluble in neutral (aqueous) ethanol Purified glutelin is a heterogeneous protein with molecules ranging in weight from 20,000 to more than 1,000,000 (*15*) Like zein, the smaller molecules have an axial ratio of about 20 to 1 (*15*)

Since lysine is the most limiting essential amino acid in corn it is of interest to compare the lysine content of various fractions obtained by the copper method (*2*). The acid-soluble and "glutelin" fractions of germ contain 6.4 and 5.8% of lysine in the proteins, respectively, and these two fractions together represent 90 to 95% of the nitrogen (*2*). The acid soluble and glutelin fractions of the endosperm contain 2.9 and 2.2% of lysine in the proteins, respectively. Thus, the acid soluble and glutelin fractions of the endosperm contain less lysine than the corresponding fractions of the germ. These data represent values from composite samples but nevertheless should indicate comparative values among the fractions. As expected, the zein of endosperm contained the lowest level of lysine found in any fraction (0.08%).

Mutant Gene Changing Protein Composition of Endosperm

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Copper fractionation (*21*) of protein from *opaque 2* endosperm revealed 15.7% zein and 42.3% glutelin based on total protein (*17*). Endosperms from normal North American and Guatemalan maize lines (*2*) contained 41 to 52% zein and 17 to 28% glutelin on the same basis. Thus there is a reversal in the ratio of zein to glutelin in the *opaque 2* endosperm when compared with normal maize lines. Two additional strains containing the *opaque-2* gene in different genetic backgrounds from each other, and from the *opaque 2* first tested, also had lysine levels twice that found in normal endosperms.

To make a critical test of the hypothesis that the *opaque 2* mutant is responsible for the increased lysine content the normal and *opaque-2* kernels from a single backcross ear ($+/o_2 \times o_2/o_2$) were separated (*18*). The amino acid composition of the defatted acid hydrolyzed endosperms is shown in Table III. Both types of endosperms contain 8.69% crude protein (N × 6.25) on a fat and moisture free basis. Thus the amino acid contents are directly comparable.

The *opaque 2* endosperm (Table III) contains 69% more lysine than the normal endosperm. The former contains less glutamic acid, alanine, leucine, and tyrosine, and more lysine, histidine arginine aspartic acid, and glycine than the latter. These changes have been confirmed in

Table III Amino Acids in Normal and Opaque Endosperms from S Ear of Corn

(Grams per 100 g protein)

Amino Acid	*Endosperm*	
	Opaque	*Normal*
Lysine	3 39	2 00
Histidine	3 35	2 82
Amide ammonia	3 41	3 28
Arginine	5 10	3 76
Aspartic acid	8 45	6 17
Glutamic acid	19 13	21 30
Threonine	3 91	3 48
Serine	4 99	5 17
Proline	9 36	9 67
Glycine	4 02	3 24
Alanine	6 99	8 13
Valine	4 98	4 68
Cystine	2 35	1 79
Methionine	2 00	2 83
Isoleucine	3 91	3 82
Leucine	11 63	14 29
Tyrosine	4 71	5 26
Phenylalanine	4 96	5 29

several different genetic backgrounds homozygous for the *opaque-2* (see Tables VI and VII)

Copper fractionation *(21)* of duplicate 0 5-gram portions of gr defatted *opaque-2* endosperm, and of a single 0 5 gram portion of n endosperm from the same ear of corn used in Table III gave the foll distributions based on soluble nitrogen *Opaque-2* 35% acid-soluble, alcohol soluble (zein), and 39% alkali-soluble (glutelin), normal acid-soluble, 37% alcohol-soluble (zein), and 29% alkali-soluble (glut This confirms the reduction in the zein-glutelin ratio observed previ *(17)*

Data on the amino acid content of the above separated soluble c fractions show that along with the reduction in the zein to glutelin important changes occur in the amino acid patterns of the acid-solubl alcohol-soluble (zein) fractions *(19)* Table IV shows that all three amino acids are increased markedly in the acid-soluble fraction of *opa* endosperm Using the *opaque-2* values as numerators and the nc values as denominators, the ratios are lysine 3 2, histidine 3 4, arginine 2 3 The *opaque-2* acid-soluble fraction also contains a ı higher level of aspartic acid, and a lower level of leucine

Table IV also shows that the major chan e in the alcohol-so (zein) fraction is a marked increase in lysi level is 3 3 times

found in the normal endosperms from the same ear of corn. However, the "normal" endosperms carry the *opaque-2* gene as a recessive, which may cause a slight elevation of the lysine level. The lysine level in the *opaque 2* zein (Table IV) is more than ten times the amount (0.08 gram) found in a composite of zein fractions from U.S. and Guatemalan endosperms. The higher level of lysine found in *opaque 2* zein isolated by copper fractionation may be a consequence of the method of fractionation. Mossé (personal communication), and Bates, Concon, and Jimenez (personal communication) found no difference in the lysine contents of zeins extracted from *opaque 2* and normal maize endosperm with alcohol by the Osborne method.

Table IV shows that only minor differences in amino acid composition exist between the alkali-soluble (glutelin) fractions of *opaque 2* and normal endosperms.

The embryos of the kernels used in Tables III and IV were defatted, acid hydrolyzed, and analyzed in the same manner as the endosperms (*19*). Table V shows that only minor differences exist between the embryos of *opaque 2* and normal kernels. Both contain a relatively high level of lysine (about 6%). The effect of the *opaque 2* gene is therefore primarily (or perhaps exclusively) confined to the endosperm.

Table IV. Amino Acids in Protein Fractions

(Grams per 100 g. protein)

Amino Acid	*In Acid-Soluble Fractions*		*In Alcohol-soluble Fractions*		*In Alkali-Soluble Fractions*	
	Opaque	*Normal*	*Opaque*	*Normal*	*Opaque*	*Normal*
Lysine	5.9	1.8	1.0	0.3	3.7	3.6
Histidine	6.5	1.2	2.1	1.6	3.8	4.2
Ammonia	2.8	3.1	4.5	2.5	1.9	2.2
Arginine	10.0	2.8	3.1	2.5	5.8	5.3
Aspartic acid	7.4	4.8	5.8	6.0	6.8	6.9
Glutamic acid	15.9	15.7	23.5	26.0	12.9	14.6
Threonine	2.3	2.4	3.4	3.4	3.3	3.4
Serine	3.7	3.9	5.6	6.5	3.7	3.7
Proline	9.9	10.9	10.5	10.5	8.7	10.1
Glycine	3.1	2.6	2.6	2.1	3.3	3.8
Alanine	4.8	5.4	8.8	10.2	4.5	4.7
Valine	2.4	2.4	4.2	4.5	5.4	5.6
Cystine	—	—	—	—	—	—
Methionine	0.8	1.4	1.6	2.5	1.4	1.1
Isoleucine	2.2	3.0	4.2	4.2	3.4	3.4
Leucine	7.4	10.1	18.8	20.3	8.1	8.6
Tyrosine	2.8	3.4	4.8	5.4	2.9	3.1
Phenylalanine	3.2	3.2	7.1	7.1	3.8	3.8

On the basis of these findings, the increased content of lysine in the *opaque-2* endosperm can be attributed to three factors increased lysine in the acid-soluble fraction, increased lysine in the zein fraction, and reduction in the ratio of zein to glutelin

Table V Amino Acids in Maize Embryos

(Grams per 100 g protein)

Amino Acid	*Opaque*	*Normal*
Lysine	5 9	6 1
Histidine	2 9	2 9
Ammonia	2 1	2 2
Arginine	9 2	9 1
Aspartic acid	9 2	8 2
Glutamic acid	13 9	13 1
Threonine	3 7	3 9
Serine	5 0	5 5
Proline	5 3	4 8
Glycine	5 5	5 4
Alanine	5 8	6 0
Valine	4 4	5 3
Cystine	0 9	1 0
Methionine	1 5	1 7
Isoleucine	2 5	3 1
Leucine	5 6	6 5
Tyrosine	2 2	2 9
Phenylalanine	3 6	4 1

A Second Mutant Gene Affecting Amino Acid Composition of Maize Endosperm Proteins

In the 1963 survey of mutant varieties, a strain homozygous for the recessive mutant gene, *floury-2* (fl_2), had a lysine content (3 2% of the endosperm protein) intermediate between that of normal maize and *opaque-2* maize Further examination of this mutant was postponed until the preliminary examination of *opaque-2* selections could be completed A comparison was then made of the amino acid patterns of the endosperms of five different mutants of similar phenotype, *opaque 1* (o_1), *opaque-2* (o_2), *floury-1* (fl_1), *floury-2* (fl_2), and soft starch (h) The data (*26*) in Table VI compare the amino acid levels in a normal (nonmutant) inbred and the five mutants investigated The normal inbred was W64A The *opaque-2* (o_2) stock analyzed here was a mutant that occurred spontaneously in the W64A stock and presumably differs from W64 only at the *opaque-2* locus Apart from *opaque-2*, only *floury 2* has a major effect on amino acid levels For *floury-2*, two analyses of the same line are given One sample was grown in 1958 and analyzed in 1964 the other grown in 1964 and analyzed in 1965 It is apparent that the level of lysine in

Table VI Amino Acid Levels for Endosperm Mutants

(Grams per 100 g protein)

Amino Acid	W64A +	W64A o_2	o_1	fl_1	fl_2	fl_2[b]	h
Lysine	1.6	3.7	1.7	1.8	3.2	3.7	1.8
Tryptophan	0.3	0.7	—	—	—	—	—
Histidine	2.9	3.2	2.3	3.2	2.0	2.6	2.7
Ammonia	4.3	3.3	3.7	3.1	2.4	4.2	4.3
Arginine	3.4	5.2	3.3	3.7	4.6	4.6	3.8
Aspartic acid	7.0	10.8	6.1	4.9	7.4	11.8	7.0
Threonine	3.5	3.7	3.3	3.1	3.0	3.9	3.6
Serine	5.6	4.8	4.9	4.7	4.2	5.8	5.7
Glutamic acid	26.0	19.8	21.5	20.2	17.5	22.2	23.7
Proline	8.6	8.6	9.4	11.9	7.6	10.8	10.4
Glycine	3.0	4.7	2.9	2.8	3.0	4.0	3.4
Alanine	10.1	7.2	8.2	7.7	7.3	9.3	9.4
Cystine	1.8	0.9	2.2	—	2.0	1.7	1.5
Valine	5.4	5.3	4.7	4.4	4.8	6.1	5.0
Methionine	2.0	1.8	2.2	2.5	3.0	3.7	2.8
Isoleucine	4.5	3.9	4.2	3.8	3.8	4.6	4.3
Leucine	18.8	11.6	15.4	15.1	12.7	15.0	16.8
Tyrosine	5.3	3.9	5.0	4.9	4.3	5.1	5.6
Phenylalanine	6.5	4.9	5.7	5.3	4.8	5.9	6.2
% protein	11.1	7.5	10.4	10.8	13.6	12.6	10.8

[a] 1958 crop
[b] 1964 crop
Analyzed by the Spies-Chambers method (*23*) by J. M. Concon

floury 2 is approximately twice that of *normal*, *opaque-1*, *floury-1* or *soft starch* and nearly as high as *opaque 2*. The protein content of this *floury 2* line is higher than the *opaque 2* line with which it is compared. Further, *floury 2* has a higher methionine level (3 to 3.7 grams per 100 grams of protein) than any line (normal or mutant) tested heretofore.

The enhanced lysine level in *opaque-2* stocks results in part from the production of zein with a greater percentage of lysine (as discussed above). Preliminary investigations indicate that the lysine level of *floury-2* zein (0.3 gram per 100 grams of zein) is only one third that found in *opaque 2* zein. In addition starch gel electrophoresis of *floury-2* zein gives a pattern like that of normal zein and different from that of *opaque-2* (*13*). Apparently the biochemical basis of elevated lysine level in *floury 2* is different from that in *opaque 2*, raising the definite possibility that in the double mutant stocks now being derived in our breeding program there may be higher lysine levels than in either mutant alone.

Growth of Rats Fed on Opaque 2 Maize

Based on chemical composition, the protein of *opaque 2* endosperms should be of greater food value than that of normal endosperms. Suffi-

On the basis of these findings, the increased content of lysine in t *opaque-2* endosperm can be attributed to three factors increased lysi in the acid-soluble fraction, increased lysine in the zein fraction, a reduction in the ratio of zein to glutelin

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(Grams per 100 g protein)

Amino Acid	*Opaque*	*Normal*
Lysine	5 9	6 1
Histidine	2 9	2 9
Ammonia	2 1	2 2
Arginine	9 2	9 1
Aspartic acid	9 2	8 2
Glutamic acid	13 9	13 1
Threonine	3 7	3 9
Serine	5 0	5 5
Proline	5 3	4 8
Glycine	5 5	5 4
Alanine	5 8	6 0
Valine	4 4	5 3
Cystine	0 9	1 0
Methionine	1 5	1 7
Isoleucine	2 5	3 1
Leucine	5 6	6 5
Tyrosine	2 2	2 9
Phenylalanine	3 6	4 1

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(Grams per 100 g protein)

Amino Acid	W64A +[a]	W64A o_2[b]	o_1	fl_1	fl_2	fl_2'	*h*
Lysine	1.6	3.7	1.7	1.8	3.2	3.7	1.8
Tryptophan	0.3	0.7	—	—	—	—	—
Histidine	2.9	3.2	2.3	3.2	2.0	2.6	2.7
Ammonia	4.3	3.3	3.7	3.1	2.4	4.2	4.3
Arginine	3.4	5.2	3.3	3.7	4.6	4.6	3.8
Aspartic acid	7.0	10.8	6.1	4.9	7.4	11.8	7.0
Threonine	3.5	3.7	3.3	3.1	3.0	3.9	3.6
Serine	5.6	4.8	4.9	4.7	4.2	5.8	5.7
Glutamic acid	26.0	19.8	21.5	20.2	17.5	22.2	23.7
Proline	8.6	8.6	9.4	11.9	7.6	10.8	10.4
Glycine	3.0	4.7	2.9	2.8	3.0	4.0	3.4
Alanine	10.1	7.2	8.2	7.7	7.3	9.3	9.4
Cystine	1.8	0.9	2.2	—	2.0	1.7	1.5
Valine	5.4	5.3	4.7	4.4	4.8	6.1	5.0
Methionine	2.0	1.8	2.2	2.5	3.0	3.7	2.8
Isoleucine	4.5	3.9	4.2	3.8	3.8	4.6	4.3
Leucine	18.8	11.6	15.4	15.1	12.7	15.0	16.8
Tyrosine	5.3	3.9	5.0	4.9	4.3	5.1	5.6
Phenylalanine	6.5	4.9	5.7	5.3	4.8	5.9	6.2
% protein	11.1	7.5	10.4	10.8	13.6	12.6	10.8

[a] 1958 crop
[b] 1964 crop
Analyzed by the Spies Chambers method (2[illegible]) by J. M. Concon.

floury 2 is approximately twice that of *normal*, *opaque-1*, *floury-1*, or *soft starch* and nearly as high as *opaque 2*. The protein content of this *floury 2* line is higher than the *opaque 2* line with which it is compared. Further, *floury 2* has a higher methionine level (3 to 3.7 grams per 100 grams of protein) than any line (normal or mutant) tested heretofore.

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Growth of Rats Fed on Opaque 2 Maize

Based on chemical composition the protein of *opaque 2* endosperms should be of greater food value than that of normal endosperms. Suffi-

Table VII Amino Acids in Opaque 2 and Normal Defatted Corn[a]

(Grams per 100 g protein)

Amino Acid	*Opaque 2*	*Normal Corn*
Lysine	4 7	2 8
Histidine	3 0	3 0
Ammonia	2 7	3 4
Arginine	6 5	4 8
Aspartic acid	10 6	6 7
Glutamic acid	17 9	20 8
Threonine	3 9	3 6
Serine	4 9	4 8
Proline	8 1	10 0
Glycine	4 8	3 8
Alanine	6 9	7 9
Valine	5 5	5 0
Cystine	1 4	1 2
Methionine	1 9	2 0
Isoleucine	3 8	4 0
Leucine	9 8	13 9
Tyrosine	3 6	4 0
Phenylalanine	4 8	5 2

Values for trytophan not available

cient amounts of *opaque-2* maize were harvested in the fall of 1964 to permit two feeding tests with rats (*23*, *24*) Table VII shows the amino acid composition of the *opaque-2* and normal maize used in the second feeding test (*23*) The *opaque-2* maize was from a backcross progeny and the whole seed contained 1 69% nitrogen The plants were self-pollinated in order to exclude pollen from normal plants The normal or nonopaque maize was a standard hybrid, Indiana 453, grown in a yield trial in which pollination was not controlled The whole seed contained 1 68% nitrogen For each 16 grams of nitrogen, the ground, defatted whole kernel of *opaque 2* maize contained 4 7 grams of lysine and the standard hybrid, 2 8 grams of lysine (Table VII) The lysine values, which are higher than those obtained on the endosperm only (see Tables III and VI), reflect the presence of the embryo, which contains approximately 6 grams of lysine for each 16 grams of nitrogen (see Table V) With the exception of histidine, the differences in amino acid composition of the whole kernel (Table VII) are similar to those observed in mutant and normal endosperms from the same ear of maize (Table III)

In the second feeding trial (*23*), groups of six weanling rats (Wistar strain), each weighing 40 to 57 grams were kept in individual wire-mesh cages, and given unrestricted access to one of the following diets diet A, 90% *opaque-2* maize, 5% corn oil, 4% Hawk-Oser salt mixture No 3,

and 1% of Vitamin Fortification Mixture (General Biochemicals), diet B, the same as diet A except that Indiana hybrid 453 replaced *opaque 2* maize diet C 10% casein, domestic, 75% starch, 10% corn oil, 4% Hawk-Oser salt mixture No 3, and 1% Vitamin Fortification Mixture, diet D, the same as diet C but containing 20% of high quality heat treated soybean meal, no casein, and 65% corn starch

The results of feeding the rats on these diets are shown in Table VIII In spite of special feed cups scattering was a problem in all groups, with the greatest incidence in the group fed on hybrid maize (diet B) Protein efficiency ratios (PER) were calculated for all animals, but the average PER values include only animals which did not scatter food, or scattered only slightly Howe Jansen and Gilfillan (*12*) surveyed the PER values of nine unsupplemented ground whole cereal grains fed to rats on diets containing 7 8 to 10% protein, and found oats to have the highest value (1 8) when compared with casein (2 5) Barley rice, maize, bulgar, rye, wheat sorghum and millet were below oats in PER The feeding tests summarized in Table VIII suggest that in young rats *opaque 2* maize proteins have a food value equal to that of heat treated soybean meal and superior to any cultivated cereal grain

Diets A and B were labeled with 1% metallic oxide, and the digestibility of the proteins was measured as described by Mertz Rennert, and Cole (*22*) The digestibility of the maize proteins was approximately 85% in both diets (*23*), this indicates that better digestibility of proteins does not appear to be a factor contributing to the growth promoting properties of *opaque 2* maize

In the first feeding test with the same number of rats (*24*), the average gain in weight for 28 days was 86 grams for rats fed a different strain of *opaque 2* maize and 23 grams for rats fed Indiana hybrid 257, a 3 7-fold difference In the second feeding test (*23*), a 3 6 fold difference was observed (Table VIII) Figure 1 shows average weekly gains of the animals on diets A and B (Table VIII) Similar curves were obtained in the first feeding test (*24*)

The greater efficiency of *opaque-2* maize endosperm proteins in rats provides a basis for assuming that they would also be superior to ordinary maize proteins in the diet of man and domestic animals To date the limited quantities of *opaque 2* maize have restricted feeding tests to the white rat, but we expect to have several thousand pounds of this mutant available in the fall of 1965 Feeding experiments with weanling pigs and chicks as well as with children and adults have been planned for the 1965-66 school year Sufficient amounts of the *floury 2* mutant should also be available in the fall of 1965 to permit feeding tests with rats

Table VII Amino Acids in Opaque-2 and Normal Defatted Corn*

(Grams per 100 g protein)

Amino Acid	*Opaque 2*	*Normal Corn*
Lysine	4 7	2 8
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Ammonia	2 7	3 4
Arginine	6 5	4 8
Aspartic acid	10 6	6 7
Glutamic acid	17 9	20 8
Threonine	3 9	3 6
Serine	4 9	4 8
Proline	8 1	10 0
Glycine	4 8	3 8
Alanine	6 9	7 9
Valine	5 5	5 0
Cystine	1 4	1 2
Methionine	1 9	2 0
Isoleucine	3 8	4 0
Leucine	9 8	13 9
Tyrosine	3 6	4 0
Phenylalanine	4 8	5 2

Values for trytophan not available

cient amounts of *opaque-2* maize were harvested in the fall of 1964 to permit two feeding tests with rats (*23*, *24*) Table VII shows the amino acid composition of the *opaque-2* and normal maize used in the second feeding test (*23*) The *opaque 2* maize was from a backcross progeny and the whole seed contained 1 69% nitrogen The plants were self pollinated in order to exclude pollen from normal plants The normal or nonopaque maize was a standard hybrid, Indiana 453, grown in a yield trial in which pollination was not controlled The whole seed contained 1 68% nitrogen For each 16 grams of nitrogen, the ground, defatted whole kernel of *opaque 2* maize contained 4 7 grams of lysine and the standard hybrid, 2 8 grams of lysine (Table VII) The lysine values which are higher than those obtained on the endosperm only (see Tables III and VI), reflect the presence of the embryo, which contains approximately 6 grams of lysine for each 16 grams of nitrogen (see Table V) With the exception of histidine, the differences in amino acid composition of the whole kernel (Table VII) are similar to those observed in mutant and normal endosperms from the same ear of maize (Table III)

In the second feeding trial (*23*), groups of six weanling rats (Wistar strain), each weighing 40 to 57 grams were kept in individual wire mesh cages, and given unrestricted access to one of the following diets diet A, 90% *opaque-2* maize, 5% corn oil, 4% Hawk-Oser salt mixture No 3,

Table VIII Gains in Weight, Protein Consumption, and Protein Efficiency Ratios (28 Days)

Animal No	*Gain, G*	*Protein Eaten, G*	*PER*[a]	*Animal No*	*Gain, G*	*Protein Eaten, G*	*PER*[a]
Diet Containing Casein				*Diet Containing Indiana Hybrid 453*			
C-1	70	20 3	3 4	B-13	32	38 0[b]	0 8[b]
C-2	54	17 2	3 1	B-14	33	19 8	1 7
C-3	72	31 6[b]	2 3[b]	B-15	23	21 5[b]	1 1[b]
C-4	73	21 0	3 5	B-16	23	14 3	1 6
C-5	43	17 4[c]	2 5[c]	B-17	23	19 7[b]	1 2[b]
C-6	85	26 7	3 2	B-18	30	19 5	1 5
Av	66		3 1	Av	27		1 6
Diet Containing Opaque 2 Maize				*Diet Containing Soybean Meal*			
A-7	86	30 0	2 9	D-19	67	35 6[b]	1 9[b]
A-8	102	38 0[b]	2 7[b]	D-20	95	34 0	2 8
A-9	134	41 6	3 2	D-21	95	36 0	2 6
A-10	81	30 7	2 6	D-22	101	37 2	2 7[c]
A-11	96	37 0	2 6	D-23	78	28 7	2 7
A-12	85	38 2[b]	2 2[b]	D-24	105	37 3	2 8
Av	97		2 8		90		2 7

[a] Protein efficiency ratio (grams gained divided by grams protein eaten) Percentage of protein (N × 6 25) in diets diet C, 8 1 diets A and B, 9 5 diet D 10 0
[b] *Value not included in average because of food scattering*
[c] Rat scattered food slightly but value included in average

Incorporation of Opaque 2 Gene into High Protein Maize Lines

In preliminary studies *(23)*, we found that the *opaque-2* gene exerts its effect in a high protein background, and selection is feasible for lines that are high in both lysine and protein The ratio of zein to glutelin in the endosperm of high protein maize is reduced when the maize is homozygous for the *opaque-2* gene In one example, the level of zein in the endosperm was reduced from 75 to 45% *(19)* Opaque kernels in high protein background *(23)* gave endosperms containing 19 2% protein, 2 7% of which was lysine Nonopaque kernels on the same ear yielded endosperms with 19 2% protein, 1 3% of which was lysine It is reasonable to assume that *opaque 2* maize containing 20% protein and 3 grams of lysine per 100 grams of protein can be produced One hundred and fifty grams of this maize should provide the minimum daily lysine requirement of most 70 kg adults *(4)* Whether it would also provide the daily requirement for tryptophan and total nitrogen is not known at present The data in Table VI show that *opaque-2* endosperm contains twice as

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Discussion

M L Anson The discovery of a variety of corn with much more lysine and tryptophan than usual corn is, as everybody must realize, of historic importance not only because of the prospect of getting corn of greatly enhanced protein nutritional value but because of the stimulus it will surely give to the research for better varieties, protein-wise, of other protein crops

It [illegible] to be undertaken as soon as [illegible] the total protein contents [illegible] varieties of all the major [illegible] accompanied by research on [illegible] of the essential amino acids [illegible] It is important that non [illegible] The opaque corn [illegible] commercial varieties.

Wh[illegible] program it would be small [illegible] reward. Grains with [illegible] offer great and unique advantages [illegible] generally [illegible] for the fight on protein [illegible] available for the fight on [illegible] of buying time is worth any [illegible]

[illegible] Howe [illegible] I believe this is the most important [illegible] It is generally agreed that [illegible] important problem. If some means cannot be found [illegible] the rate of population increase, nothing [illegible] will have any appreciable long term effect.

The second most important [illegible] the energy requirements of the people of the [illegible] Raymond Ewell of the University of Buffalo [illegible] our being able to do this. He predicts famine [illegible] in the 1970's. Others are more optimistic. H[illegible] it will be accomplished largely by more [illegible] of cereal grains by using improved agricultural te[illegible] increased use of fertilizer and higher yielding hybrid strains. M[illegible] the people of the underdeveloped countries will have to [illegible] entirely on cereal grains. What then will be their protein intake? S[illegible] calculations show that if the energy requirements of man [illegible] of his development are supplied entirely by cereal, his protein intake will be adequate in quantity, even with rice which has the lowest protein content, but not in quality, since kwashiorkor, a protein-deficiency disease, occurs in some children who subsist entirely on cereal.

A great deal has been made of the fact that recent experiments show that adult men can be maintained in nitrogen balance on a diet consisting almost *entirely of cereal grains. Why this finding should receive such* emphasis is perplexing since in the underdeveloped countries many men live out their lives on a predominantly cereal diet. Whether they would be better off with a higher quality protein is not known. Whether the better health enjoyed in a country such as ours can be attributed partially to our

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It seems self-evident that there ought to be undertaken as soon as possible an immense crash program to analyze the total protein contents and the amino acid compositions of all available varieties of all the major grains, oilseeds, and pulses. This should be accompanied by research on easier, quicker methods for the estimation of the essential amino acids, preferably without hydrolysis of the proteins. It is important that noncommercial as well as commercial varieties be studied. The opaque corn and the original cotton with gland-free seed are noncommercial varieties.

Whatever the cost of the immense crash program, it would be small compared with the possible, if not very likely, reward. Grains with improved protein content and composition offer great and unique advantages for animal and human nutrition in general and for the fight on protein malnutrition in particular. The time available for the fight on human malnutrition is so short that the possibility of buying time is worth any effort, any cost, any risk.

E. J. Howe: Related to the world food supply, I believe this is the most important development since the intrauterine device. It is generally agreed that population control is the most important problem. If some means cannot be found for greatly decreasing the rate of population increase, nothing we do concerning food supply will have any appreciable long-term effect.

The second most important problem is to satisfy the energy requirements of the people of the developing countries. Raymond Ewell of the University of Buffalo is very pessimistic about our being able to do this. He predicts a famine of unprecedented magnitude in the 1970's. Others are more optimistic. However, if famine is prevented, it will be accomplished largely by increasing the production of cereal grains by using improved agricultural technology, such as increased use of fertilizer and higher yielding hybrid strains. Most of the people of the underdeveloped countries will have to subsist almost entirely on cereal grains. What then will be their protein intake? Simple calculations show that if the energy requirements of man in any stage of his development are supplied entirely by cereals, his protein intake will be adequate in quantity, even with rice which has the lowest protein content, but not in quality, since kwashiorkor, a protein-deficiency disease, occurs in some children who subsist entirely on cereals.

A great deal has been made of the fact that recent experiments show that adult men can be maintained in nitrogen balance on a diet consisting almost entirely of cereal grains. Why this finding should receive such emphasis is perplexing since in the underdeveloped countries many men live out their lives on a predominantly cereal diet. Whether they would be better off with a higher quality protein is not known. Whether the better health enjoyed in a country such as ours can be attributed partially to our

intake of high quality protein or entirely to better sanitation, freedom from parasites, etc , is open to question Certainly, until it is shown that high quality protein is not beneficial to adults, it would seem reasonable for them to consume it if it can be made economically available

This, however, is not the problem The problem is in the nutrition of the children Children who receive enough of their mothers' milk to supply caloric requirements do not develop kwashiorkor A certain percentage of those subsisting on cereals do develop this disease, even though the total protein intake is as great or greater than that obtained from mothers' milk It follows, therefore, that if a child received an adequate caloric supply from cereals, he would, if the protein of the cereal were comparable with milk protein, almost certainly be free from protein malnutrition Mertz's work has shown that a corn can be grown, whose protein is comparable to casein Moreover, steps have already been taken to introduce the high quality protein gene into high yielding hybrid varieties

It is certainly much better to induce nature to add the necessary components to cereal proteins to improve their protein quality than to add man-made amino acids While for technical reasons it is more difficult to obtain high quality protein-containing cereals other than corn, unquestionably it can be done It is strongly urged that all available plant breeders be mobilized to attack this problem, the solution of which would go far to preventing protein deficiency in the vast cereal-eating areas of the world I hope this important development by Mertz and his associates will be given the emphasis it deserves

Safflower, A Potential Source of Protein for Human Food

G O KOHLER

Western Regional Research Laboratory Western Utilization Research and Development Division U S Department of Agriculture Albany Calif

Safflower has been grown for centuries in the Nile Valley and in parts of Asia It is a newly established U S crop and is expected to become a major one In dry climates where adequate soil moisture is available it yields over a ton of seed per acre, rich in high quality oil and protein Chick experiments indicate that the meal is relatively free of physiologically deleterious components and properly supplemented produces high growth rates The relatively high content of sulfur amino acids and low content of lysine suggest combinations with soybean protein Preliminary work has yielded low fiber palatable foods More research is needed to develop the potential of safflower as a source of human food

The critical nature of the problem of feeding an expanding world population large segments of which are already undernourished has been documented thoroughly (*2 28 29*) Since both calorie and protein deficits present primary problems it is logical to look to the oilseeds as major direct food sources of the future because oilseeds are rich in both calories (as oil) and protein

During the past decade a new oilseed crop safflower (*Carthamus tinctorius* L) has been developing at a healthy rate in the United States Initially grown as a source of industrial oil, broader markets are developing for the oil as a food oil Research on the protein rich meal indicates that safflower has promise as a major source of protein for direct food use as well as a source of calories A useful bibliography on safflower is available (*17*) Reviews of production processing and utilization of safflower have been published (*11, 13*) and a symposium on safflower was presented at the 1965 meeting of the American Oil Chemists Society (*1*)

Although it is a new commercial crop in the United States, safflower is actually one of the oldest of the cultivated plants. It has been grown since ancient times in the Nile Valley, the Middle East, and the Orient, primarily as a source of the dyestuff, carthamin—for example, some of the linen wrapping cloths of the mummies of Egypt were dyed with safflower flower extracts (*9, 33*). Commercial types of safflower are thistle like annual plants which grow from 1½ to 4 feet in height and produce light yellow to deep orange flower heads (Figure 1), each of which contains 15 to 50 white seeds resembling sunflower seeds in shape but somewhat smaller.



Figure 1. Safflower flowers. Left: Seed head with flower. Right: Cut in half to show developing seeds. Courtesy of P. F. Knowles, University of California, Davis, Calif.

The plant is adapted to dry climates such as are found in the Central Valleys of California and extensive plain areas from Arizona and New Mexico to southwestern Canada. Yields in unirrigated areas average 350 to 1500 pounds of seed per acre. On irrigated land, yields are in the range of 1800 to 3000 pounds per acre. Experimental and commercial yields as high as 2½ tons per acre have been recorded, showing that the productive capacity of the crop is extremely high (*13*).

Although safflower was introduced to this hemisphere by early Spanish mmigrants (*12*), no serious effort was made to develop it as an oilseed crop til the introduction of new seeds and beginning of research by the U. S. partment of Agriculture in 1925. Outstanding in the early research

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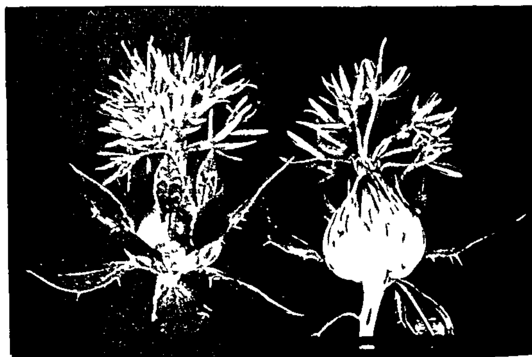


Figure 1 Safflower flowers Left Seed head with flower Right Cut in half to show developing seeds Courtesy of P F Knowles, University of California, Davis Calif

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effort was the [illegible] its chemurgic project in the 1930's and early 1940's.

Although several abortive attempts were made to develop commercial supplies prior to 1948, successful development of the crop started when production was begun in California where there was a good environment for the crop and strong support from a few oilseed processors. The subsequent growth in annual production shown in Figure 2, was based on development of improved disease-resistant, high-oil varieties, on improved agronomic practices, on development of industrial uses for the oil through technological research (24), and on increasing export markets for the seed, largely in Japan. The large spurt in 1963 reflects the entry of safflower oil into the food market. Riding the crest of public interest in the blood cholesterol-depressing effects of polyunsaturated oils, new producers and processors swelled production of safflower oil as the polyunsaturated oil par excellence (80% linoleic acid). The resultant overproduction and temporary imbalance between production and marketing were serious but are now history and, regardless of the medical significance of blood cholesterol lowering effects, resumption of the steady growth pattern is expected, with safflower oil selling at prices competitive with corn or cottonseed oil.

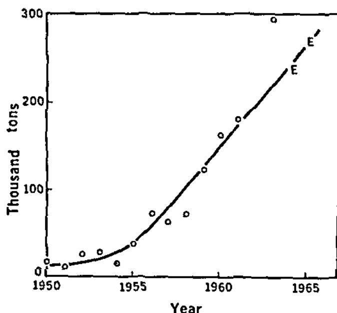


Figure 2. U. S. production of safflower seed

At present the United States produces about one half of the world supply of safflower seed which was roughly estimated at 640,000 tons in 1964. The second and third largest producers of safflower seed are

India and Mexico, which produce about 750,000 and 60,000 tons, respectively Successful experimental crops in Australia and Venezuela may lead to expanded production in these and other countries (*30*)

Early varieties of safflower seed contained 49 to 52% hull (*20*) Typical seeds of present commercial varieties are made up of 55 to 65% kernel and 35 to 45% hull (*15*) The whole seeds contain about 35 to 40% of oil and about 13 to 17% of protein Almost all of the oil and protein are located in the kernel, so that a completely dehulled or decorticated kernel contains about 60% of oil The completely decorticated, oil-extracted kernel contains about 60 to 70% of protein Figure 3 shows the products theoretically obtainable from 1 ton of seed Safflower has wide genetic variability, and plant breeders are well along in the development of thin-hulled gray, brown-striped, and white varieties which contain only half as much hull as present commercial types These new types of seed contain proportionately higher levels of oil and protein and can be expected to replace the present types (*18*, *27*) Types of safflower seeds have also been found, the oils of which are strikingly different from the safflower oil of commerce, e g , oils containing up to 75% oleic acid (*14*) These new types give promise of expanded broader uses of safflower oils with the accompanying increased supply of protein

Table I compares the essential amino acids of safflower kernel protein (*24*) with those of 50% protein soybean meal and with the FAO recommended provisional amino acid reference pattern (*7*) The data on safflower and soybean meal were obtained by the method of Moore and Stern (*21*), using correction factors established for safflower protein for

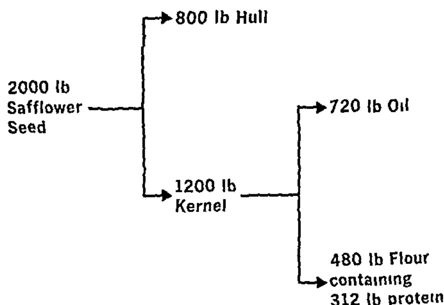


Figure 3 Theoretical yields of products from one ton of safflower seed

Table I Safflower Seed and Soybean Proteins Compared with FAO Provisional Reference Amino Acid Pattern

	Provisional Pattern G/16g N		Safflower			Soybean		
	Orig	Adjusted	G/16 g N	% of Pattern Orig	% of Pattern Adjusted	G/16 g N	% of Pattern Orig	% of Pattern Adjusted
Isoleucine	4.2		4.0	95	95	4.8		
Leucine	4.8		6.2			7.3		
Lysine	4.2		3.1	74	74	5.8		
Phenylalanine	2.8		4.4			4.8		
Tyrosine	2.8		3.1			3.0		
Total sulfur AA	4.2	3.4	3.3	79	97	2.9	68	85
Methionine	2.2	1.7	1.7	78		1.4	64	83
Threonine	2.8	3.3	3.3			3.8		
Tryptophan	1.4	1.1	1.6			1.7		
Valine	4.2	2.8	5.7			5.0		

Chemical score is per cent of pattern for first limiting amino acid

the labile amino acids (threonine, serine, and tyrosine) and for the difficultly released amino acids (isoleucine and valine). By applying the fluorodinitrobenzene method (26) it was found that the apparent availability of the lysine in safflower flour was 100%. The first limiting amino acid of safflower is lysine. However, methionine is limiting to about the same degree based on the 1957 FAO reference pattern. More recent results on human requirements (10, 23) suggest that several of the figures for individual amino acids in the original pattern are too high and in one case too low. The numbers under "provisional pattern" adjusted in Table I refer to adjusted FAO pattern figures for four of the amino acids in question. Using the adjusted FAO reference value for methionine, safflower is limited only by lysine and isoleucine and methionine are borderline. Soybean protein shows a lower chemical score than safflower based on the 1957 pattern. Reducing the methionine in the adjusted pattern raises the score of soybean meal since methionine is the first limiting amino acid.

Another comparison of safflower and soybean, with egg protein as a reference protein is shown in Table II. This is calculated as suggested in the 1965 FAO/WHO report (10). Safflower protein is comparable to soybean protein in ratio of essential to total amino acids (E/T value) and in score. When lysine is added to the safflower, its "egg reference" score goes up to about 85, and methionine and isoleucine become about equally limiting. Based on the adjusted 1957 reference pattern a combination of ⅓ safflower and ⅔ rice protein would have a chemical score of 81, higher than either one alone but still limited by both lysine and me-

Table II Comparison of Safflower and Soybean Proteins
(Egg protein as reference pattern)

	Egg	*Safflower*	*Soybean*
Ratio g. essential amino acids per g. N (E/T)	3.22	2.17	2.08
Chemical score based on egg protein	100	71	70
Limiting amino acid	—	Lysine	Methionine

thionine. By combining safflower protein with soybean on a 3 to 2 basis, a score of 93 is obtained. Thus safflower and soybean proteins show excellent mutual supplementation. Similarly, milk, fish, pulses, and other protein sources rich in lysine will adequately supplement safflower (*3*).

A comparison of safflower and soybean protein from the standpoint of amino acids not included in the provisional pattern is shown in Table III. Safflower contains adequate amounts of histidine to meet the needs of children. It is a rich source of glycine and arginine, which are essentials for the chick.

Since safflower shows great genetic variability in fatty acid composition, seed shape and color, hull thickness, etc., we wondered if amino acid composition might also be controlled by breeding. In a very preliminary study (*24*) of some 20 widely different seed types, we found that none of the essential amino acids varied more than about 7% from the mean. More research is needed along these lines, including tests of individual plants from widely divergent sources.

Literature reports on biological evaluation of safflower protein are very sparse. According to Baliga *et al.* (*3, 16*) the biological values

Table III Amino Acids Not in FAO Provisional Reference Amino Acid Pattern

	Amino Acid, G./16 G. N	
	Safflower	*Soybean*
Histidine	2.4	2.5
Arginine	9.3	6.9
Glycine	5.8	4.0
Aspartic acid	9.8	10.6
Glutamic acid	19.4	17.6
Serine	4.4	5.1
Proline	4.1	5.2
Alanine	5.8	4.1
Total (% of protein)	61.0	56.0

(BV) of safflower protein of two samples of safflower meal were 84.9 and 86.0%. The coefficient of true digestibility was 92.4 for a 35.7% protein sample and 76.5 for a 21.8% protein sample. The only protein efficiency ratio (PER) value found in the literature was 1.3 for hydraulic pressed safflower cake (22).

Our results, adjusted to the casein standard (4), on two samples of commercial partially decorticated meal were 1.40 and 1.26, respectively. PER value of a sample of pilot plant produced meal from brown striped thin hulled seed and a sample of laboratory produced unheated safflower flour were 1.24 and 1.35 respectively. Supplementation of the laboratory-produced 58% protein safflower flour with methionine alone (Table IV) raised the PER only slightly. Adding lysine as well raised the PER to 2.09. In other experiments adding lysine without methionine had little effect on PER. Thus the PER assays verify the chemical score estimates based on rat requirements and show that lysine and methionine are almost equally limiting amino acids for the rat.

Table IV Effect on PER of Supplementation of Safflower Flour with Methionine or Methionine Plus Lysine

	Cystine plus Methionine G/16G N	*Lysine G/16G N*	*PFR*
Safflower flour (58% protein)	3.31	3.09	1.39
Same plus methionine	*4.20*	*3.07*	*1.59*
Same plus methionine and lysine	4.20	5.00	2.09

Since the amino acid balance appeared excellent when methionine and lysine were added, we set up chick experiments to determine whether methionine-supplemented safflower meal could provide the basis for a bioassay of available lysine. Quadruplicate equalized groups of seven 5-day-old broiler type chicks were fed rations devised to be complete in all known required nutrients except lysine. Partially decorticated safflower meal (44% protein) supplemented with methionine, was used as the primary source of protein with corn or glucose as the primary energy source. Graded levels of L-lysine were added. The results given in Figure 4 show that the types of dose response curves obtained were satisfactory for assay purposes.

The results show two other important points. First, even when safflower protein is fed at levels high enough to supply over 93% of the chicks protein intake—e.g. glucose safflower ration—there is no evidence of any inhibitors such as are found in unheated soybeans and in raw cottonseed meal. Second, at optimum levels of lysine addition safflower consistently produces faster growth than optimally treated soybean meal. Further data substantiating this observation are shown in Table V. In

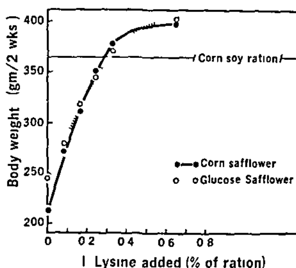


Figure 4 Dose response curves for chicks for lysine supplementation of safflower meal-based rations

all cases safflower meal produced better rates of gain than did soybean meal Fisher *et al* (*6*) showed a similar growth effect of safflower during the course of net protein value (NPV) assays with chicks In this study the safflower-fed chicks grew better than soybean fed chicks, even though the NPV for safflower supplemented with methionine and lysine was only 46 2 as compared with 68 8 for methionine-supplemented, decorticated soybean meal (*6*) It does not seem likely that the effect is due to amino acid imbalance in soybean protein since attempts to increase growth by amino acid supplements to the soybean ration have been unsuccessful More research will be needed to determine whether this difference between safflower and soybean meals is due to the presence of inhibitors in the soybean meal, the presence of an unidentified growth factor in the safflower meal, or some ration imbalance in the soybean protein not immediately apparent

The high fiber content of safflower meal makes it unacceptable as a protein source for human foods It is generally considered that high protein products, at least for children, should contain less than 5% fiber Hull has not been separated commercially from kernel to produce such products from safflower For the past several years one company has been selling a partially decorticated safflower meal containing 42% protein for use in poultry and swine rations This product contains about 15% fiber and is thus undesirable for a human food

We have been able to prepare products with 57 to 60% protein and about 3% fiber from thin-hulled seeds by an experimental laboratory process based on air separation, roller milling, and screening operations (*8*)

Although a great deal more laboratory and development research will be required to develop a commercially feasible process, the difficulties do not appear insurmountable. The products obtained by this experimental process were very light colored but had a bitter taste. Preliminary experiments showed that practically all of the bitter principle could be extracted with acetone or alcohol with less than 5% loss of nitrogen. The washed product, which contained about 70% protein, had a rather characteristic mild flavor which was not deemed undesirable by an informal taste panel. Further preliminary tests showed that meat-like patties containing this 70% protein safflower flour were acceptable to the panel. When the product was added to a bread at a 5% level based on the flour, loaf volume was reduced only slightly and a detectable but not unpleasant flavor was noticeable. More extensive tests are in progress.

Other promising routes to human products lie in preparation of protein isolates (22) and in preparation of textured products (5) from safflower. Here again, considerably more research will be needed to bring preliminary results to commercial usage.

Table V. Growth of Chicks Fed Safflower as Compared with Soybean Meal[c]

Protein in Ration, %	*Duration of Experiment, Weeks*	*% Increase*
22	2	6.4
	2+	6.8
	2+	6.3
	2+	12.2
	2+	10.0
	3	3.7
	3+	4.0
	4	17.8
	4	6.0
20	2	3.3
18	2	17.6
	2+	18.6

[a] With added methionine and lysine.
[b] With added methionine.
[c] $\frac{\text{Weight gain on safflower meal} - \text{weight gain on soybean meal}}{\text{weight gain on soybean meal}} \times 100$

Conclusions

Safflower is a rapidly expanding commercial oilseed crop which may well become a major world crop. It is adapted to semiarid lands of the types found in many protein-deficient areas of the world. It is already

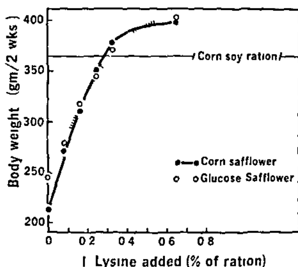


Figure 4 Dose response curves for chicks for lysine supplementation of safflower meal-based rations

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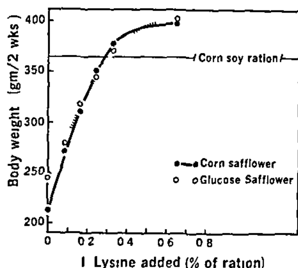


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a high yielding plant, but its genetic variability gives promise of still further improvements through breeding It produces high yields of edible oil as well as protein The protein is highly nutritious if properly supplemented with lysine Its relatively high content of sulfur amino acids and low lysine content suggest its use in combinations with soybean protein Preliminary work has shown that conversion to palatable food products is possible, although much research and development work are needed

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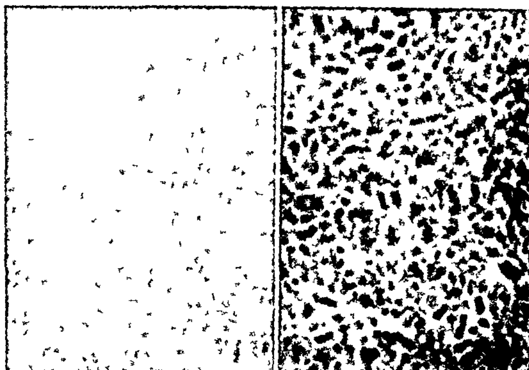


Figure 2 Disruption of B megaterium cells (× 1000)
Left Cell suspension of B megaterium (ca 10% dry weight)
Right Suspension at same concentration after homogenization at 8000 to 10,000 p s i

is no significant interference in this method from compounds such as nucleic acid, glucosamine, or *N*-acetylglucosamine

The cell wall fraction constituted a large proportion of the cell (57% by weight) and contained a high percentage of the total cell amino acids (Table III) No further protein could be extracted from this fraction with

Table III Amino Acid Content of Cell Fractions of *B megaterium*

	% N	*% Protein by Biuret*	*% Amino Acids*	*% of Total Cell Amino Acids*
Whole cells	6 3	—	38	—
Water-soluble fractions				
TCA precipitate		68	68	15
HCl precipitate		77	—	—
Buffer-soluble fraction				
TCA precipitate		100	52	37
HCl precipitate		99	—	—
Cell wall fraction	5 3	—		48

salt solutions over a wide pH range Preparation of purified cell walls generally entails digestion with a number of different enzymes The cell wall preparation from *B megaterium* reported by Salton (*9*) represented 20 to 25% of the weight of the cell, and contained 5 3% nitrogen In this case assuming an even distribution of amino acids throughout this fraction, it would appear that a minimum of approximately 25% of the amino acids in the cell would be unavailable

Table IV Amino Acid Composition of Whole Cells and Water Soluble Fraction of *B megaterium*
(G /16 g nitrogen)

Amino acid	*Whole Cells*	*Water-Soluble Fraction*	*Cow's Milk (9)*
Arginine	6 7	5 4	3 7
Histidine	2 3	2 3	2 7
Lysine	6 3	8 6	7 9
Leucine	11 0	9 3	10 0
Isoleucine	4 4	6 3	6 5
Methionine	3 1	2 6	2 5
Cystine		1 0	0 9
Phenylalanine	5 4	4 9	4 9
Tyrosine	5 0	3 8	5 1
Threonine	4 8	4 3	4 7
Valine	6 5	6 6	7 0
Aspartic acid	9 1	9 2	
Glutamic acid	18 9	13 7	
Serine	3 4	3 9	
Proline	[b]	3 1	
Glycine	4 0	3 8	
Alanine	10 1	5 7	

Not measurable
[b] Not determined

The amino acid compositions of *B megaterium* whole cells and of the water-soluble fraction of these cells are compared in Table IV with that of whole cow's milk protein The results on whole cells are not in good agreement with those of Garibaldi *et al* (*6*) who were able to account for only 50 to 60% of total nitrogen by microbiological assay The possible presence of large amounts of D amino acids in the cells might account for part of this difference Anderson and coworkers (*2*) reported 11 8% lysine and 3 4% methionine in *B megaterium* using microbiological assay, indicating that differences among various workers may be due to the particular strain of the organism studied

The results in Table IV do not show large differences in essential amino acid content between the whole cells and the water soluble fraction of *B megaterium* and cow's milk protein, with the possible exception of a

low value for lysine in whole cells As previously discussed, the values for whole cells may be misleading because of the probable unavailability of some of the amino acids The final nutritional evaluation of whole cells, disrupted cells, and protein fractions derived from the cell will have to await comparative feeding studies in animals The present availability of a method for disrupting large quantities of cells will make this study possible

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19

Fungi and World Protein Supply

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In view of the magnitude of the problem of producing additional protein for a rapidly increasing population, it is suggested that certain of the fungi imperfecti can make large contributions to the world protein pool by converting carbohydrate and inorganic nitrogen to protein On the bases of results of experiments conducted with cane molasses sugar beets, potatoes, sweet potatoes manioc corn and rice projections were made which indicate that sufficient protein for a world population of 4 5 billion can be produced by conversion of the carbohydrates of these seven crop plants to fungal protein

Although the rapid rate of human population increase has created many serious problems the major immediate problem which man has created for himself through the increase of his species is a nutritional one and specifically is that of producing ever increasing amounts of nutritionally adequate protein in high enough concentration to meet the requirements of an expanding population At the present rate of increase world population now becomes larger by approximately 55 million people annually From this, and the fact that each individual should be supplied with about 52 pounds of protein annually we can easily illustrate the magnitude of the problem confronting us If present population trends continue, the world is faced with the staggering problem of producing 2 8 billion more pounds of protein each year than it produced the previous year Such an expansion of protein production cannot continue indefinitely, but perhaps it could be achieved for a period long enough to institute an effective crash educational program designed to establish the concept that population regulation measures must be established on a world wide basis How long such a program would require before this concept is generally accepted cannot be estimated at this time However it is safe to predict that many years will be required since countries with a high percentage of illiteracy or those which adhere strictly to Marxian concepts cannot be expected to accept such views quickly

Although the United States is now in a favorable position with respect to protein, in the long-term view its problem may be greater than that of countries in which the eating habits are different from those of this country Since our protein of choice is animal protein, far greater amounts of vegetable protein must be produced in this country than in countries which have the same rate of population increase but in which the protein consumed directly by the human population is primarily vegetable rather than animal Assuming that eating habits remain unchanged, to meet the requirements of our additional U S citizens we must increase meat production by the equivalent of an additional million head of beef animals each year, which demands the production of far greater amounts of vegetable protein to be used for conversion to animal protein

Two other aspects of the protein problem must be considered In addition to total quantity of protein, attention must be paid to concentration of protein in materials used for food, and quality of protein must also be considered In the work presented here, only quantity and concentration are considered, although the importance of protein quality is fully recognized

Barring a major breakthrough in the area of protein chemistry, it is highly improbable that any single program or process will provide a complete answer to the protein problem However, if significant contributions to the world protein pool can be made by several different methods, the spread of protein deficiency may be halted until world wide population regulation is a reality

Fungi as Protein Synthesizers

Since many fungi are rapidly growing organisms and can synthesize their amino acids and hence their protein directly from carbohydrate and inorganic nitrogen compounds, a program was initiated in the Mycology Laboratories of the Ohio State University in 1960 to explore certain fungi as potential synthesizers of protein since unlike animal protein, such protein would not be formed inefficiently and at the expense of previously synthesized vegetable protein In September of 1964 this project was transferred to Southern Illinois University, where it still continues

The idea of using microorganisms is not new (*3*), and man has undoubtedly eaten fungi for many centuries However fungi have rarely been viewed as a staple item of the diet and for that reason and in view of the present population problem it seems necessary to call attention again to the potential resident in this type of organism Although much lay opinion and other folklore mitigate strongly against acceptance of the concept of placing fungi in widespread usage as a food source there are no valid reasons why many could not be eaten There are a number of

poisonous fungi, but so are there poisonous species in every major group of living organisms however, such species represent a small minority What seems to be a natural antipathy for considering fungi as food is reflected in the mushroom industry in the United States—in spite of the fact that there are several hundred species of edible fleshy fungi, only one species is produced commercially

In selecting fungi for use in the present project, only members of the fungi imperfecti were considered, because members of this group are numerous ubiquitous and rapid growing The initial work has been described (*6*, *7*) and need not be repeated here except to list the criteria which were used to form the basis for selection of fungi to be used

The fungus must be capable of utilizing inorganic nitrogen as a sole source of nitrogen

It must be able to grow well in submerged culture

It must grow rapidly 4 days were arbitrarily set as the maximum acceptable period for growth

It must efficiently convert substrate carbon to tissue carbon and at the same time convert inorganic nitrogen to organic nitrogen

Initial screening experiments involved culturing many imperfect fungi on a standard medium which permitted growth (although it was not necessarily optimum) but did permit comparison of yields and judgments as to how well the above criteria were met A surprising number of fungi were found suitable although as might be expected, some performed better than others Results of some of the screenings reported by Gray, Och, and AbouГlSeoud (*8*) illustrate that of the first 175 fungi tested, over 50% had economic coefficient values of 3 00 or less, and 7 4% had values of 2 01 or less in other words they were capable of producing 1 unit weight of dried fungus mycelium from 2 01 or less unit weights of glucose

$$\text{Economic coefficient} = \frac{\text{grams dry weight of sugar utilized}}{\text{grams dry weight of fungus produced}}$$

Percentages of crude protein of the 18 fungi which yielded best in the above series ranged from 13 4 to 30 8 in other experiments crude protein percentages as high as 38% have been obtained Subsequent work has shown that both economic coefficient values and protein percentages can be improved by proper alteration of environmental conditions

Once the feasibility of the process was established by demonstration that 1 unit weight of crude fungus protein could be synthesized during the utilization of 5 to 8 unit weights of glucose the work was expanded to include the use of crude carbohydrate-containing waste products and plant materials Thus far white potatoes, sweet potatoes sugar beets, manioc cassava flour, rice corn, citrus molasses beet molasses blackstrap molasses beet shreds, wood pulp, and wood flour have been considered With the

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exception of the last three, all of the above crude raw materials can be used satisfactorily as a source of carbohydrate for the synthesis of fungus protein, and the last three can probably be used successfully if further investigated

With few exceptions these fungi produce spherical mycelial pellets in submerged liquid culture These pellets vary in size with species, amount of aeration and agitation, and size of inoculum and are usually white or nearly white in glucose medium but may have the color of the substrate when cultured on such materials as ground sweet potato Freshly harvested mycelial pellets are odorless or have a faint mushroom odor but when dried are virtually odorless and tasteless They may be dried and ground to a flour, made into flakes, or pressed into tablets

Imperfect fungi have been found which can synthesize 1 unit weight of crude protein from 6 unit weights of sugar Since protein values were based upon Kjeldahl nitrogen × 6 25, they are subject to the same criticism as all such values so derived Furthermore, the cell walls of the imperfect fungi are chitinous, and the amount of chitin varies not only with species (*2*) but with age (*1*) Therefore our crude protein values are probably high, and hence the following projections which are concerned with the synthesis of fungal protein from crude raw materials are conservatively based upon the assumption that 1 unit weight of protein will be formed in the course of 8 unit weights of carbohydrate being utilized

Manioc

Grown throughout the tropical and subtropical world, manioc is a staple item of diet in many such areas The long fleshy roots contain 32% carbohydrate but only 0 7% protein, hence protein deficiency is widespread in manioc-eating areas Assuming that manioc protein is complete (which it is not), an individual would have to eat about 24 pounds of fresh roots daily to obtain his required 65 grams of protein As carbohydrate sources in fungus growth medium we have used minced, fresh, whole roots as well as the crudely ground native cassava flour which is available in such areas as coastal West Africa, while both materials are suitable sources, fresh roots were found to be the better substrate Since yields from fresh roots are generally higher, the following calculations are based on this raw material

World production of manioc during the 1962/63 season was 75 5 × 10^6 metric tons (*4*), which contained 54 144 × 10^9 pounds of carbohydrate Assuming that the proper fungus (*Cladosporium*, No 83, which has grown so well experimentally on manioc) will synthesize 1 pound of protein from 8 pounds of carbohydrate, it would be possible to produce 6 768 × 10^9 pounds of protein a 5 7-fold increase in protein over and above that initially present (1 18 × 10^9 pounds) in the manioc Thus, over six times as much

protein can be produced from the same yield from the same area on the same acreage. Since under the usual conditions of tropical agriculture manioc yields are not nearly so high as they might be, a conservative estimate would be that yield could be tripled with no great difficulty. A tripling of yield followed by a fungal conversion process could result in the production of an additional 22 676 × 10⁶ pounds of protein over that currently produced on the same land by manioc cultivation alone. Assuming that this protein is complete (which it probably is not but could be supplemented) it would supply the total annual protein requirements for an additional 434 million people.

Rice

This cereal grain is nutritionally far more adequate as a food source than manioc since it contains about 70% carbohydrate but more importantly 7.5% protein. In the 1962/63 season world production of paddy rice was 247.4 × 10⁶ metric tons which contained 387 023 × 10⁶ pounds of carbohydrate and 41 563 × 10⁶ pounds of protein. Using I–108 (*Dactylium dendroides*) or I–193 (*Trichoderma* sp.) it would be possible to produce an additional 18 49 × 10⁶ pounds of protein. Thus it would be possible to produce a total quantity of protein (rice plus fungus) sufficient (on purely quantitative grounds) to meet the annual requirements of 1 713 × 10⁶ people. Since yields of rice per unit area of land in Japan greatly exceed those of India, world yields of rice can be greatly increased by improving rice cultivation in India.

Sugar Cane and Sugar Beets

Man's major source of sucrose has traditionally been sugar cane grown in tropical and subtropical areas but as the demand grows more and more is being produced in temperate regions from sugar beet. In the 1962/63 season cane was harvested from over 18 × 10⁶ acres and beet from over 16 × 10⁶ acres so on this basis it would appear that there is nearly equal production of the two crops. If we assume
of 40 tons (containing 12% sugar) and of beets of 18 tons (co
sugar) per acre we find that the total cane crop contained
pounds of sugar compared with 92 16 × 10⁹ pounds in th
However most cane is an 18 month crop, and if cane
is calculated on an annual basis it was 115 2 × 10⁹ pounds
with 92 16 × 10⁹ pounds of beet sugar. Thus from the
duced in one year by cane and beets 25 92 × 10⁹ pounds
be produced by a fungus conversion process using I–75
To this may be added the 4 32 × 10⁹ pounds present
protein is not included because of its low concentr

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With few exceptions these fungi produce spherical mycelial pellets in submerged liquid culture These pellets vary in size with species, amount of aeration and agitation, and size of inoculum and are usually white or nearly white in glucose medium but may have the color of the substrate when cultured on such materials as ground sweet potato Freshly harvested mycelial pellets are odorless or have a faint mushroom odor but when dried are virtually odorless and tasteless They may be dried and ground to a flour, made into flakes, or pressed into tablets

Imperfect fungi have been found which can synthesize 1 unit weight of crude protein from 6 unit weights of sugar Since protein values were based upon Kjeldahl nitrogen $\times$ 6 25, they are subject to the same criticism as all such values so derived Furthermore, the cell walls of the imperfect fungi are chitinous, and the amount of chitin varies not only with species (*2*) but with age (*1*) Therefore our crude protein values are probably high, and hence the following projections which are concerned with the synthesis of fungal protein from crude raw materials are conservatively based upon the assumption that 1 unit weight of protein will be formed in the course of 8 unit weights of carbohydrate being utilized

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- Cane and Sugar Beets

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Although the United States is now in a favorable position with respect to protein, in the long-term view its problem may be greater than that of countries in which the eating habits are different from those of this country. Since our protein of choice is animal protein, far greater amounts of vegetable protein must be produced in this country than in countries which have the same rate of population increase but in which the protein consumed directly by the human population is primarily vegetable rather than animal. Assuming that eating habits remain unchanged, to meet the requirements of our additional U.S. citizens we must increase meat production by the equivalent of an additional million head of beef animals each year which demands the production of far greater amounts of vegetable protein to be used for conversion to animal protein.

Two other aspects of the protein problem must be considered. In addition to total quantity of protein, attention must be paid to concentration of protein in materials used for food, and quality of protein must also be considered. In the work presented here, only quantity and concentration are considered, although the importance of protein quality is fully recognized.

Barring a major breakthrough in the area of protein chemistry, it is highly improbable that any single program or process will provide a complete answer to the protein problem. However, if significant contributions to the world protein pool can be made by several different methods, the spread of protein deficiency may be halted until world wide population regulation is a reality.

Fungi as Protein Synthesizers

Since many fungi are rapidly growing organisms and can synthesize their amino acids and hence their protein directly from carbohydrate and inorganic nitrogen compounds, a program was initiated in the Mycology Laboratories of the Ohio State University in 1960 to explore certain fungi as potential synthesizers of protein since, unlike animal protein, such protein would not be formed inefficiently and at the expense of previously synthesized vegetable protein. In September of 1964 this project was transferred to Southern Illinois University, where it still continues.

The idea of using microorganisms is not new (*3*), and man has undoubtedly eaten fungi for many centuries. However, fungi have rarely been viewed as a staple item of the diet, and for that reason and in view of the present population problem it seems necessary to call attention again to the potential resident in this type of organism. Although much lay opinion and other folklore mitigate strongly against acceptance of the concept of placing fungi in widespread usage as a food source, there are no valid reasons why many could not be eaten. There are a number of

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quantity of protein the potential exists for producing far more than is now required for the current world population

Concentration and Quality of Fungus Protein

From the standpoint of protein concentration no special problems would be encountered with fungus mycelium since the majority of fungi we have examined, even on standard medium which is not optimum, have crude protein contents of 20% or higher. Furthermore, protein content can usually be increased by proper manipulation of environmental conditions.

With regard to fungus protein quality we are still in a great unknown area. In short term mice-feeding experiments with I-9 (*Heterocephalum aurantiacum*) as the sole source of food, adult mice maintained their weight and weanling mice showed as great a weight gain as controls on commercial mice food. Such feeding trials will have to be repeated, lengthened, and expanded to include many other species. Unfortunately, most of the nutritional studies using mold mycelia have been conducted with species of *Aspergillus* and *Penicillium*. Thus Skinner (*9*) used *P. flavo-glaucum*, Takata (*11*) used *A. oryzae*, Skinner *et al.* (*10*) used *A. fischeri*, *A. oryzae*, and *P. chrysogenum*, and Woolley and his associates (*13*) used *A. sydowi*, and all found the proteins of these fungi to be inadequate. Foster (*5*), presumably on the basis of the above reports, states that mold proteins

Conversion of Carbohydrate of Seven Crop Plants to Protein, Agents of Biosynthesis

Total Crop Protein Billion Lb	*Protein Available By Fungus Conversion Billion Lb*	*Total Protein Billion Lb*	*Factorial Increase in Protein*	*No of People Who Can Be Supplied with 52 2 Lb /Yr, Millions*
1 184	6 768	7 952	6 71 ×	152
4 320	11 520	15 840	3 66 ×	305
—	14 400	14 400	—	276
41 563	48 490	90 053	2 19 ×	1725
34 094	36 540	70 634	2 07 ×	1350
4 705	9 149	13 854	2 94 ×	265
11 772	14 057	25 829	2 19 ×	494
97 638	140 924	238 562	—	4567

of 30 24 $\times 10^9$ pounds of protein—sufficient, on quantitative grounds, to meet the annual requirements of 581 million people

Other Crops

Experimental work has also been conducted with minced white potatoes, minced sweet potatoes, and ground corn Projections based upon results obtained with these materials are presented in Table I along with those discussed above On the basis of results obtained so far it is a safe prediction that any cereal grain or any high carbohydrate fleshy plant part could be used in a project such as that described, although use of each material would entail a search for the most suitable fungus and optimum conditions From the calculations summarized in Table I, it is obvious that during the 1962/63 season sufficient protein was produced in six crop plants (cane is omitted because of its low protein content) to provide 52 2 pounds of protein for each of 1 87 billion people However, if the carbohydrate of these six crop plants plus the carbohydrate of cane had been used as a carbon source for the culture of imperfect fungi, sufficient additional protein could have been produced to provide 52 2 pounds of protein for each of an additional 2 697 billion people In other words, from these seven crop plants, sufficient protein could have been produced to meet the annual needs (in so far as quantity is concerned) of 4,567,000,000 people—a figure approximately 50% greater than the world population in early July of 1962 Thus, with respect to total

Table I World Protein Production Potential (1962/63) Based on Using Fungi as

Crop	*World Production (4) 1962/63, Million Tons*	*Carbohydrate, %*	*Total Carbohydrate, Billion Lb*	*Protein, %*
Manioc	84 600	32	54 144	0 7
Sugar beet	288 000	16	92 160	0 75
Sugar cane	480 000	12	115 200	—
Paddy rice	277 088	70	387 923	7 5
Corn	243 600	60	292 320	7 0
Yams and sweet potatoes	130 704	28	73 194	1 8
Potatoes	294 324	19	112 434	2 0
Totals	1798 316	—	1127 375	—

20

Microbial Synthesis of Food From Coal-Derived Material

MELVIN P. SILVERMAN,[1] JOAN N. GORDON, and IRVING WENDER

Pittsburgh Coal Research Center, Bureau of Mines, U. S. Department of the Interior, Pittsburgh, Pa.

Cell yields of four strains of the yeast Candida grown on n-hexadecane ranged from 487 to 719 mg. dry weight per gram of hydrocarbon added; growth on 1-octadecene decreased about one half. A normal paraffin fraction from petroleum gave yields of 244 to 4[illegible]0 mg.; three Fischer-Tropsch fractions and one low temperature coal tar fraction gave yields within this range. Bacteria, but not yeasts, grew on three coal-acid mixtures and a synthetic mixture of polynuclear aromatic hydrocarbons with yields below 100 mg. In general, high growth yields of yeasts were favored by substrates that were high boiling, contained a high ratio of normal paraffins to olefins, and were low in phenols. The crude protein content of Candida grown on a Fischer-Tropsch fraction was 41.1%.

The problem of the world's population explosion is compounded by an over-all global food shortage. New or unusual sources of food, especially high-quality protein, are needed as dietary supplements for both humans and animals. The Permanent Section on Food Microbiology and Hygiene, International Association of Microbiological Societies, called for an increased contribution of microbiology to world food supplies in a unanimous resolution outlining several research areas, including hydrocarbon microbiology, which might lead to increased world food production (14).

Some recent reports have dealt with the microbial conversion of petroleum hydrocarbons to protein, vitamins, or amino acids. High yields of yeast cells rich in protein and vitamins have been obtained at the expense of the n-alkanes (preferably C_{10} or higher) in crude petroleum

[1] Present address: Ames Research Center, Exobiology Division, National Aeronautics & Space Administration, Moffett Field, Calif.

are inadequate to satisfy the protein requirements of animals wholly—an exceptionally broad statement in view of the fact that less than a fraction of 1% of the fungi have ever been tested On the other hand, Vinson *et al* (*12*) found that mycelia of *Fusarium lini* (if supplemented with thiamin) and *F graminearum* (if supplemented with multiple B vitamins) were satisfactory sources of protein for normal growth, reproduction, and lactation in mice

In view of the vast numbers of species and strains of imperfect fungi that exist, it would seem that this is a very fruitful area to explore There is a very real possibility that some strains will be poisonous, and, like the proteins of green plants, some will be more nutritionally adequate than others It is not suggested that by themselves the fungi will solve all of the world's protein problems, however, it is time to dispel some of the myths of mycology and have a much closer look at a group of living organisms which appear to have a very real potential in the area of protein synthesis

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Table I Analysis of Fischer Tropsch (SASOL) Fraction

Hydrocarbon		*FIA Analysis*	
Carbon No	%	*Compounds*	%
C [illegible]	2	Aromatics	Nil
C [illegible]	11	Olefin	33
C [illegible]	17	Paraffins*	64
C [illegible]	16	Oxygenates	3
C [illegible]	15		
C [illegible]	13		
C [illegible]	11		
C [illegible]	9		
C [illegible]	4		
C [illegible]	2		

* Normal Hydrocarbons over 90%

from 1000 hour nitric acid oxidation of anthracite by the Bureau of Mines' Anthracite Research Center (7)

Substrate CIA a synthetic mixture of polynuclear aromatic hydrocarbon *found in relatively large amounts in high temperature coal tar,* consisted of 1 methylnaphthalene 59 2 2 methylnaphthalene, 16 3, naphthalene 11 9 and phenanthrene 12 5 wt %

Microorganisms and Culture Media Cultures were obtained from soil by standard enrichment culture techniques or from the culture collections of the University of Pittsburgh Syracuse University and the University of Iowa

Basal medium N was prepared by adding NH_4NO_3 (5 0 grams), K_2HPO_4 (2 5 grams), and $MgSO_4$ $7H_2O$ (1 0 gram) to 1 liter of tap water The pH was adjusted to 7 0 and any insoluble salts were removed by filtration The clear medium was sterilized by autoclaving Medium NY was prepared by adding sufficient filter-sterilized yeast extract to medium N to make a final concentration of 0 01%

Measurements of Growth Yields Yeast inocula were prepared from cultures grown overnight in 50 ml of Mycophil broth (Baltimore Biological Laboratory Baltimore Md) The resultant growth was collected by centrifugation washed twice in sterile medium N and resuspended in 20 ml of the same solution One milliliter of washed yeast-cell suspension was the standard inoculum for all experiments

For studies on growth yield of yeasts these standard inocula were added to 50 ml of medium NY in 300-ml Erlenmeyer flasks in triplicate A quantity of substrate equivalent to 0 3 ml was weighed into each flask Controls consisted of triplicate inoculated flasks without added substrate Cultures were incubated at 30°C on a rotary shaker (225 r p m) for 6 days The resultant growth was collected on 2 inch diameter Alpha 8 Metricel filters of 0 20 μ pore size (Alpha Metricel, a regenerated cellulose (rayon) membrane is a product of the Gelman Instrument Co , Ann Arbor, Mich) The growths were washed with 10-ml volumes of acetone and

fractions (*2–6*), feedstocks (*13, 16*), or the pure hydrocarbons themselves (*1, 4, 9, 12*) Microbial synthesis of amino acids from petroleum products has also been reported (*16, 19*) Early papers discussed the use of Kogasin, a synthetic fuel derived from coal, for microbial substrates (*8, 10, 11*)

The rate of microbial synthesis of protein far exceeds the rate at which animals synthesize protein A cow weighing 500 kg when fed by grazing can synthesize 0 5 kg of protein per day (*18*), whereas 500 kg of microorganisms growing on paraffinic hydrocarbons could synthesize 1250 kg of protein per day (*3*) According to one estimate, 3 million tons of protein per year (equal to the world's present protein deficit) could be produced by microorganisms at the expense of only 1% of the world's annual production of 700 million tons of crude paraffinic petroleum (*3*)

Since coal is one of the world's cheapest and most abundant sources of fixed carbon, we investigated the question whether coal, like petroleum, could be converted by microorganisms into high-protein food This report gives the results of a comparative evaluation of coal-derived materials, pure hydrocarbons, and petroleum-derived material as raw materials for microbial production of food The use of coal-derived liquid fractions was emphasized because of the intriguing possibility of extending the utility of fuel and chemical products obtained from coal conversion processes

Experimental

Materials Three fractions of Bureau of Mines Fischer-Tropsch synthetic liquid fuel (iron catalyst) were used fraction FTL (boiling range 0 to 204°C), fraction FTD (boiling range 204 to 316°), and fraction FTW (boiling point > 316°) The analysis of Fischer-Tropsch fraction SASOL (from the South African Coal, Oil, and Gas Corp) is given in Table I

Two fractions of hexane-soluble material from Rockdale lignite low-temperature tar were obtained from the Texas Power and Light Co hexane-soluble foreruns (HSF) and a hexane-soluble distillate (HSD) The HSF fraction constituted 7%, and the HSD fraction 46% of the primary tar The compositions of fractions HSF and HSD are given in Table II Phenolic compounds were removed by chromatographing fractions HSF and HSD on alumina with petroleum ether as the eluent to obtain phenol-free fractions HSFϕ and HSDϕ About 20 weight-% of starting material was removed by this procedure

A paraffin-rich fraction (CTP) and a linear paraffin-olefin fraction (CTPO), both derived from the neutral oil of low-temperature tar, were supplied by the Bureau of Mines' Morgantown Coal Research Center Their analyses of fractions CTP and CTPO are given in Table III The normal paraffin fraction (PET) was derived from petroleum by an industrial firm Our mass spectrometric analysis of this fraction is given in Table IV

Three mixtures of coal acids were tested asg rowth substrates DOW, a 56% aqueous solution from the Dow Chemical Co , HOW, a water-soluble mixture of aromatic acids from alkaline oxidation of coal by the Carnegie Institute of Technology, and ANTH, the acid soluble residue

Table I Analysis of Fischer-Tropsch (SASOL) Fraction

Hydrocarbon		*FIA Analysis*	
Carbon No	%	*Compounds*	%
C_{11}	2	Aromatics	Nil
C_{12}	11	Olefins	33
C_{13}	17	Paraffins	64
C_{14}	16	Oxygenates	3
C_{15}	15		
C_{16}	13		
C_{17}	11		
C_{18}	9		
C_{19}	4		
C_{20}	2		

Normal Hydrocarbons ca 90%

from 1000 hour nitric acid oxidation of anthracite by the Bureau of Mines' Anthracite Research Center (7)

Substrate CTA, a synthetic mixture of polynuclear aromatic hydrocarbons found in relatively large amounts in high temperature coal tar, consisted of 1 methylnaphthalene, 59 2, 2-methylnaphthalene, 16 3, naphthalene, 11 9, and phenanthrene 12 5 wt -%

Microorganisms and Culture Media Cultures were obtained from soil by standard enrichment culture techniques or from the culture collections of the University of Pittsburgh, Syracuse University, and the University of Iowa

Basal medium N was prepared by adding NH_4NO_3 (5 0 grams), K_2HPO_4 (2 5 grams), and $MgSO_4$ $7H_2O$ (1 0 gram) to 1 liter of tap water The pH was adjusted to 7 0 and any insoluble salts were removed by filtration The clear medium was sterilized by autoclaving Medium NY was prepared by adding sufficient filter-sterilized yeast extract to medium N to make a final concentration of 0 01%

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For studies on growth yield of yeasts, these standard inocula were added to 50 ml of medium NY in 300-ml Erlenmeyer flasks in triplicate A quantity of substrate equivalent to 0 3 ml was weighed into each flask Controls consisted of triplicate inoculated flasks without added substrate Cultures were incubated at 30°C on a rotary shaker (225 r p m) for 6 days The resultant growth was collected on 2 inch diameter Alpha 8 Metricel filters of 0 20 μ pore size (Alpha Metricel, a regenerated cellulose (rayon) membrane, is a product of the Gelman Instrument Co , Ann Arbor, Mich) The growths were washed with 10-ml volumes of acetone and

Table II Approximate Composition of Rockdale Lignite Low Temperature Tar Fractions

(Volume %)

Type of Constituent	*HSF*	*HSD*
Caustic-solubles	6–8	10–15
Acid-solubles	2–4	1–3
Neutral oil	88–92	80–90
Paraffins	13–15	15–20
Olefins	40–55	40–50
Alpha-olefins	17–20	17–20
Aromatics	30–47	35–45

n-hexane, dried overnight in air, and then weighed All reported data are corrected for growth of controls

Bacterial inocula consisted of 1 ml of cultures grown for 3 days in 50 ml of medium N or NX supplemented with 1 weight-% of coal acid (DOW or HOW) or 0 5 ml of CTA Procedures for determining bacterial growth yields were similar to those for yeast cultures, except that 1 weight % of coal acid (DOW or HOW) or 0 5 ml of CTA was weighed into 50 ml of medium N or NX Incubation times were 4 days for CTA cultures and 6 days for coal acid cultures

Results

More than 200 cultures of bacteria, yeasts, and fungi were screened for viability on coal-derived materials Only bacteria (no yeasts or fungi) grew on the coal acids and CTA Conversely, yeasts and fungi produced the

Table III Analyses of Paraffin Rich (CTP) and Paraffin Olefin (CTPO) Fractions from Rockdale Lignite Low Temperature Tar

(Weight %)

	CTP		*CTPO*	
Carbon No	*n-Paraffin*	*n-Olefin*	*n-Paraffin*	*n-Olefin*
C_8	0 2	—	—	—
C_9	3 2	—	0 1	0 2
C_{10}	11 7	0 7	1 3	1 6
C_{11}	18 2	1 5	3 6	5 3
C_{12}	22 4	3 3	5 8	7 6
C_{13}	20 7	2 8	7 8	9 5
C_{14}	10 8	3 7	7 5	10 9
C_{15}	0 8	—	6 7	9 4
C_{16}	—	—	4 6	5 6
C_{17}	—	—	2 3	2 9
C_{18}	—	—	4 2	3 1
	88 0	12 0	43 9	56 1

most vigorous growth on Fischer-Tropsch, low temperature tar, and petroleum-derived fractions. Of these, the yeasts *Candida lipolytica* strains 409, 409A, 409B, and *Candida tropicalis* strain 410 were selected as the most promising cultures with respect to total cell yield and ability to grow in reasonably high concentrations of substrate.

These studies also indicated that growth yields on Fischer-Tropsch fraction FTL, low-temperature tar fractions HSI and HSI ϕ, and the coal acid ANTH were negligible. No further studies were made with these materials.

Table IV. Analysis of *n* Paraffin Fraction (PFT) from Petroleum

Carbon No	*Volume %*
C_9	0.5
C_{10}	7.2
C_{11}	37.9
C_{12}	29.1
C_{13}	23.4
C_{14}	1.9

Growth Yields of Yeasts on Pure Compounds and Coal Derived Material. Absolute growth yields in milligrams of dry weight of yield per gram of added substrate and relative growth yields are given in Table V. The highest yields for all cultures (433 to 719 mg dry weight) were obtained with *n* hexadecane as substrate. *C. lipolytica* 409 and 409A gave the best yields with *n* hexadecane. Growth yields of 300 mg or higher were obtained when 1-octadecene (*C. lipolytica* 409 and *C. tropicalis* 410) and Fischer-Tropsch fraction FTW (*C. lipolytica* 409B) served as growth substrates. Other substrates yielding more than 200 mg dry weight were paraffin rich low temperature coal tar fraction CTP (all cultures), and Fischer-Tropsch fractions FTD (*C. lipolytica* 409B) and FTW (*C. lipolytica* 409).

All cultures yielded less than 100 mg dry weight on low temperature tar fraction HSD_φ. On all substrates tested, with the exception of low-temperature tar fraction HSD_φ, *C. lipolytica* 409B consistently gave yields greater than 50% relative to yields on *n* hexadecane and appears to be the most versatile culture. *C. tropicalis* 410 is noteworthy for its ability to utilize the terminal olefin 1-octadecene (76% relative to *n* hexadecane).

Growth Yields of Yeasts on Petroleum Derived and Coal Derived Material. The results of a second experiment, comparing the absolute and relative growth yields of yeast cultures on coal-derived material with the yields on a normal paraffin fraction derived from petroleum (PFT), are given in Table VI. The three *C. lipolytica* cultures yielded over 300 mg dry

Table V Growth Yields of Yeasts on Pure Compounds, Low Temperature Tar, and Fischer Tropsch Fractions

Substrate	*C lipolytica*			*C tropicalis*
	409	*409A*	*409B*	*410*
Low temperature tar	Growth Yield			
HSDϕ[b]	83(0 12)	43(0 07)	0(0)	62(0 14)
CTP	272(0 38)	276(0 44)	294(0 68)	208(0 46)
Fischer-Tropsch				
FTD	134(0 19)	115(0 18)	280(0 65)	—
FTW	287(0 40)	127(0 20)	344(0 79)	200(0 44)
Pure compounds				
1 Octadecene	357(0 50)	295(0 47)	244(0 56)	344(0 76)
n-Hexadecane	719(1 00)	628(1 00)	433(1 00)	453(1 00)

Average of triplicate cultures Data in milligrams of dry weight of yield per gram substrate added corrected for growth in controls Values in parentheses are growth yields relative to that on *n* hexadecane

[b] Phenols removed by two passages through alumina column

weight on petroleum fraction PET Yields greater than 300 mg dry weight were also obtained on Fischer-Tropsch fraction FTW with *C lipolytica* 409 and 409B All cultures gave yields greater than 200 mg dry weight on low-temperature tar fraction CTP *C lipolytica* 409B and *C tropicalis* 410 both produced more than 200 mg of dry cells on low-temperature tar fraction CTPO *C lipolytica* 409B also yielded more than 200 mg dry weight on Fischer-Tropsch fraction FTD All cultures gave negligible or no growth on low-temperature tar fraction HSD, however, upon removal of the phenolic constituents from this fraction, *C lipolytica* 409B and *C tropicalis* 410 produced more than 100 mg of dry cell material

In terms of relative growth, *C lipolytica* 409 was outstanding on Fischer-Tropsch fraction FTW and on low-temperature tar fraction CTP, yielding 100 and 84%, respectively, of the growth obtained on PET *C lipolytica* 409A gave a relative growth yield of 71% on fraction CTP The versatility of *C lipolytica* 409B is again illustrated by relative yields ranging from 61% (fraction CTPO) to 85% (fraction FTW) on all substrates except fractions HSD and HSDϕ *C tropicalis* 410 was outstanding in its relative ability to grow on low-temperature tar fractions CTP (95%) and CTPO (84%), although absolute yields were less than those obtained with *C lipolytica* 409B on the same substrate Also interesting is the apparent correlation between the abilities of *C lipolytica* 409B and *C tropicalis* 410 to use a terminal olefin (Table V) and their ability to grow on low-temperature tar fraction CTPO, containing 56% of olefinic compounds (Table III) The *C lipolytica* 409 and 409A cultures gave poorer yields on 1-octadecene relative to *n* hexadecane (Table V) and this was reflected in their poorer absolute and relative yields on CTPO compared with CTP (Table VI)

Since the Bureau of Mines Fischer Tropsch fractions (FTD, FTW) had been stored for a long time prior to testing, confirmation of our results was sought with fresh material The new results (Table VII) demonstrated clearly the equivalence of fresh Fischer-Tropsch fraction SASOL and petroleum-derived fraction PET in terms of absolute and relative growth yields with the three *C lipolytica* strains

Pooled growth yields of five replicate cultures of *C lipolytica* 409B grown on Fischer Tropsch fraction SASOL (average yield, 151 mg of dry cell material per gram of SASOL added) were analyzed for crude protein content (Kjeldahl N × 6 25) and gave 41 1% crude protein This value compares favorably with values reported for microorganisms grown on petroleum hydrocarbons (3 6, 9, 13, 15)

Table VI Growth Yields of Yeasts on Low Temperature Tar, Fischer Tropsch, and Petroleum Fractions

Substrate	*C lipolytica*			*C tropicalis*
	409	*409A*	*409B*	*410*
Low temperature tar	Growth Yield			
HSD[b]	14(0 05)	6(0 02)	0(0)	0(0)
HSDϕ[c]	73(0 24)	72(0 22)	106(0 28)	102(0 42)
CTPO	113(0 37)	124(0 39)	233(0 61)	205(0 84)
CTP	259(0 84)	235(0 71)	287(0 75)	232(0 95)
Fischer Tropsch				
FTD	114(0 37)	150(0 45)	243(0 63)	0(0)
FTW	306(1 00)	167(0 50)	329(0 85)	161(0 66)
Petroleum				
PET	307(1 00)	333(1 00)	385(1 00)	244(1 00)

Average of triplicate cultures Data in milligrams of dry weight of yield per gram of substrate added corrected for growth in controls Values in parentheses are growth yields relative to those on PET

[b] Phenols not removed

Phenol removed by three passages through alumina column

Growth Yields of Bacteria on Coal Acids and Polynuclear Aromatic Hydrocarbons In all tests bacteria growth yields on the coal acids and on the synthetic mixture of polynuclear aromatic hydrocarbons were below 100 mg of dry cell material (Table VIII) Interestingly, most cultures produced higher cell yields in media without yeast extract (medium N)

Discussion

The over all results of our experiments are summarized in Table IX, which gives the range of growth yields obtained on the various substrates (carbon sources) with yeasts and bacteria The highest range of yields was

Table VII Growth Yields of Yeasts on Fischer Tropsch (SASOL) and Petroleum-Derived (PET) Fractions

Organism	Substrate	Experiment 1		Experiment 2	
		Mg /G	Relative yield	Mg /G	Relative yield
C lipolytica 409	PET	342	1 00	280	1 00
	SASOL	322	0 94	326	1 16
C lipolytica 409A	PET	338	1 00	306	1 00
	SASOL	347	1 03	341	1 12
C lipolytica 409B	PET	430	1 00	374	1 00
	SASOL	437	1 02	348	0 93
C tropicalis 410	PET	287	1 00	290	1 00
	SASOL	224	0 78	261	0 90

Table VIII Growth Yields of Bacteria on Coal Acids and Polynuclear Aromatic Hydrocarbons

Substrate	Microorganism	Mg Dry Wt /G Substrate Added	
		Medium N	Medium NA
Coal acids			
DOW	D 10	10	28
DOW	D 21	68	18
DOW	D 27	14	9
HOW	H 22	51	48
Polynuclear aromatics			
CTA	CTA 23	1	68
CTA	CTA 26	32	3

obtained with n-hexadecane as substrate, yields on 1-octadecene decreased by approximately one half Normal paraffin fraction PET from petroleum supported growth ranging from 244 to 430 mg dry weight Fischer-Tropsch fractions FTD, FTW, and SASOL, as well as low-temperature tar fraction CTP, supported growth falling within the range obtained with PET Growth yields on low-temperature tar fraction CTPO approached the lower limit of the yields on fraction PET The coal acids (ANTH, DOW HOW) and the mixture of polynuclear aromatic hydrocarbons (CTA) proved inferior substrates for bacteria and were incapable of supporting the growth of yeasts

The data in Table IX suggest that, in general, higher growth yields are favored by substrates which are higher boiling, and hence, of higher molecular weight (compare yields on FTL, FTD, and FTW), contain a high ratio of normal paraffins to olefins (compare yields on n-hexadecane

with 1-octadecene, PET, CTP, and SASOL with CTPO), and are low in phenols (compare yields on HSD with HSDφ).

The range of growth yields obtained with any given substrate is related in part to the inherent variability among different species or different strains of the same species and illustrates the importance of choosing the proper strain for obtaining high yields.

Table IX. Growth Yields on Pure Hydrocarbons and Fractions Derived from Coal or Petroleum

Microbe	*Substrate*	*Growth Yield, Mg./G. Substrate*
Yeasts	Low temperature Tar	
	HSF	0
	HSFφ	0
	HSD	0–14
	HSDφ[b]	0–83
	HSDφ	72–106
	CTPO	113–233
	CTP	208–294
	Fischer Tropsch	
	FTI	0
	FTD	0–280
	FTW	127–341
	SASOL	221–451
	Petroleum	
	PET	244–430
	Pure Hydrocarbons	
	1 Octadecene	241–357
	n Hexadecane	433–719
Bacteria	Coal Acids	
	ANTH	0
	DOW	9–68
	HOW	48–51
	Polynuclear Aromatics	
	CTA	1–68

Mg. of dry cell material synthesized per gram of substrate added. Low and high growth yields for each range are average yields of triplicate cultures of lowest and highest yielding cultures among cultures tested.

[b] Phenols and other material removed by two passages of HSD through alumina.

Phenols and other material removed by three passages of HSD through alumina.

Effluents *b* and *c* showed absence of hydroxyl group by infrared spectroscopy.

In practice, over 80% conversion of hydrocarbon substrates to cell material has been obtained (*1, 3, 6, 9, 13, 17*) Although our absolute growth yields on pure hydrocarbons, petroleum Fischer-Tropsch, and low-temperature tar fractions were somewhat lower, our experiments were designed solely to test the comparative feasibility of using these substrates for microbial production of food Improvements in the nutritional quality of the growth medium, closer control of pH, more efficient aeration, and other modern fermentation practices undoubtedly will result in higher cell yields

Conclusion

Paraffin-rich, higher molecular weight fractions derived from low-temperature coal tar and Fischer-Tropsch synthetic liquid fuel compare favorably with a petroleum-derived fraction as substrates (carbon sources) for microbial production of food Coal, with its enormous reserves therefore can take its place alongside petroleum as a potential source of high-protein food

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ADDENDUM. A reviewer has pointed out that some early papers appeared on the use of kogasin, a synthetic fuel derived from coal, for microbial substrates (*8, 10, 11*).

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